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Progression of vinyl chloride induced hepatic fibrosis to angiosarcoma of the liver

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ABSTRACT Two vinyl chloride monomer (VCM) workers, who developed non-cirrhotic portal fibrosis and portal hypertension, died from angiosarcoma of the liver five and ten years later respectively, despite withdrawal from occupational exposure. We suggest that non-cirrhotic portal fibrosis caused by exposure to VCM is potentially premalignant and that those workers who already have the condition should be carefully monitored.

Since the original communication by Creech and Johnson[1] there have been several further case reports of vinyl chloride monomer (VCM) induced angiosarcoma of the liver. A commoner hepatic lesion is non-cirrhotic portal fibrosis[2] leading to portal hypertension. It has been suggested that this lesion may be a precursor to angiosarcoma formation,[3–5] but there has been only one report of a patient with documented hepatic fibrosis developing angiosarcoma at a later date.[6] We describe two further cases.

Case reports

CASE 1

A 60-year old white man initially presented in 1969 to the dermatology department with a skin eruption. Examination at that time showed anaemia, thrombocytopenia, hepatosplenomegaly, and occult faecal blood loss. He had an occupational history of exposure to VCM at high concentration for seven years while working as a polycleaner and a blowdown recovery operator. After two haematemeses, barium studies and splenic venography confirmed portal hypertension and oesophageal varices, and he underwent an end-to-side portocaval shunt. At laparotomy the liver looked nodular, but operative liver biopsy showed non-cirrhotic portal fibrosis only. Over the next four years the patient developed chronic portosystemic encephalopathy and maturity onset diabetes mellitus that was treated with oral hypoglycaemic agents. In May 1980 he presented with worsening mental deterioration, hepatic foetor, asterixis, and an enlarging liver. Liver function test results showed a normal serum bilirubin with a raised alkaline phosphatase (201 IU/l) and γ-glutamyl transpeptidase (83 IU/l). Radioisotope liver scan showed a small liver with no filling defects and needle liver biopsy showed a well-pronounced micronodular cirrhosis with no evidence to tumour. He responded to protein restriction and lactulose and was discharged. Within a week of discharge he was readmitted with an endoscopically confirmed bleeding duodenal ulcer. After this, he lapsed into hepatic coma and died. Necropsy showed multiple malignant tumours in the liver (wt 1130 g), one of which was haemorrhagic. Histologically, a great variety of liver cell lesions were present, some resembling angiosarcomas, some hepatocarcinomas, and some adenomas. There were also atypical sinusoidal cells and fibrosis in the non-tumorous parts of the liver.

CASE 2

A 49-year-old man with a 12-year history of exposure to vinyl chloride monomer while working as a spray drier bagger, premix operator, and paste charging operator was, during a factory survey of process workers in 1974, found to have thrombocytopenia. This was later shown to be due to hypersplenism and presinusoidal portal hypertension. He was otherwise well and drank five pints of beer a night. Liver function test results were normal apart from a raised γ-glutamyl transpeptidase level of 82 IU/1. Varices were shown by barium studies and endoscopy, and liver biopsy showed fatty change and slight non-cirrhotic portal fibrosis. Two years later the patient developed insulin dependent diabetes mellitus, but otherwise remained well until October 1979 when he presented with a large haematemesis. Endoscopy confirmed oesophageal

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varices to be the source of haemorrhage, and these were treated by injection sclerotherapy. The serum alkaline phosphatase and γ-glutamyl transpeptidase levels rose over the next three months to 334 IU/l and 106 IU/l respectively. Radioisotope liver scan showed multiple filling defects consistent with tumour deposits. Ascites and ankle oedema developed, and he died suddenly in January 1980 from an intraperitoneal bleed after a liver biopsy. Necropsy showed that a large haemorrhagic tumour, occupying most of the right lobe of the liver, had ruptured into the peritoneum. Several smaller haemorrhagic tumours were found elsewhere in the liver. An enlarged spleen (wt 760 g) and thrombosed oesphageal varices were also noted. Histology showed the tumours to be angiosarcomas, with areas of sinusoidal dilatation and portal fibrosis in unaffected areas of the liver.

Discussion

Vinyl chloride monomer is transformed by hepatic microsomal enzymes to toxic metabolites that covalently bind to DNA.[7] After exposure to VCM hepatocytic proliferation, sinusoidal lining cell proliferation, and focal sinusoidal dilatation occur[8] and angiosarcoma may later develop from the sinusoidal lining cells. Enlarged lipocytes may be seen in the space of Disse[8]; these cells are fibroblast precursors and can lay down collagen.[9] Hepatic fibrosis results and may be associated with presinusoidal portal hypertension.[10] It is commoner than angiosarcoma.[2] Although fibrosis was found in tumour-free portions of the liver tissue from VCM workers dying of angiosarcoma,[3] transition of hepatic fibrosis to angiosarcoma, although postulated,[3] has not been observed.

The two patients described here were originally included in a series of seven VCM workers with portal hypertension.[2] They were followed for five and 10 years respectively from the time of diagnosis of portal hypertension to death from angiosarcoma. During this period they were not further exposed to VCM. Their case histories indicate that hepatic fibrosis in a VCM worker may be a precursor of future malignant change and life-long follow-up is necessary. We know of only one other similar patient who died seven years after a portacaval shunt from an angiosarcoma.[6]

Non-cirrhotic fibrosis can also occur in the absence of portal hypertension, and may then be difficult to detect. It does not lead to a disturbance of liver function tests and may even be missed on needle biopsy of the liver.[2] Greyscale ultrasonography is a useful diagnostic aid,[11] but it has yet to be used in a large industrial survey, and there are probably several unrecognised affected VCM workers. Stringent measures to control levels of exposure should lead to eventual disappearance of non-cirrhotic fibrosis. Those patients who already have the condition, however, are still at risk of developing hepatic angiosarcoma in the future.

A problem highlighted by the second case is that angiosarcoma is, by definition, a vascular lesion, and there is a risk of uncontrolled haemorrhage after blind liver biopsy. We believe, therefore, that the diagnosis should be sought either by laparoscopic biopsy or by hepatic angiography.

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ANIMAL MODEL OF HUMAN DISEASE

Hepatic Angiosarcoma

Animal Model: Angiosarcoma of Rats and Mice Induced by Vinyl Chloride

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Biologic Features

Vinyl chloride (VC, $CH_2 = CHCL$) is a monomer used mainly to make polyvinyl chloride (PVC), an essential material to our daily life. In 1974, Creech and Johnson reported hepatic angiosarcoma in workers associated with the manufacture of PVC.[1] The first case of hepatic angiosarcoma in VC workers was identified in 1961.[2] This finding was later substantiated by experimental inhalation study in laboratory animals.[3,4]

Although the definite mechanism has not been elucidated, recent animal studies have suggested that VC, like many other carcinogens, is not carcinogenic without metabolic activation.[5] Study in rodents revealed that VC was activated by cytochrome P-450 into epoxide (chloroethylene oxide), which could alkylate the macromolecules of cells and lead to neoplastic transformation. This explanation is strengthened by the findings that the exposide is a strong mutagen.[6]

Mice exposed to 50 to 1000 ppm of VC in the air for 6 hr/day, 5 days/wk, develop hepatic angiosarcoma after 6–12 months.[4] The incidence of this tumor is related to the VC level and the length of exposure. Angiosarcoma also developed in spleen, mesentery, and subcutaneous tissues. Rats exposed to the same levels of VC require a longer time to develop tumors and do not have the toxic effects seen in some mice, such as acute toxic hepatitis, tubular nephrosis, and early death in groups given higher doses. Exposure to VC followed by a 1-year recovery period did not diminish the incidence of development of these tumors.[7]

Vinyl chloride-induced hepatic angiosarcomas in mice or rats usually

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are multicentric, with no special prevalence in any lobe of the liver. Grossly, the tumors are soft, friable, and red in color. The large ones have often ruptured and formed a blood clot or filled the abdominal cavity with a blood-tinged fluid. Microscopically, the earliest recognizable neoplastic foci consist of erythrocytes admixed with a cluster of immature endothelial cells. These foci are usually located in dilated intralobular sinusoidal spaces (Figure 1). The well-developed angiosarcomas are composed of aggregates of irregular vascular channels that are lined by neoplastic endothelial cells and filled with erythrocytes (Figure 2). The neoplastic mass expands and progressively replaces the liver cords. The neoplastic cells can be divided into two types: 1) large spindle cells that resemble endothelial cells and are arranged in capillary networks and 2) large, round, or polygonal cells with no special pattern of arrangement. These cells may fuse to form giant cells. Mitotic figures are not an uncommon finding. Peliosis, extramedullary hematopoiesis, and liver necrosis are commonly associated with this tumor.

The clinical signs are not detected until late in the course of the disease. The animals with advanced angiosarcoma have anemia with generalized edema, apparently due to rupture of the tumor or leaking of blood from the neoplastic mass. The anemia could also be due to abnormal vasculature, which induces a microangiopathic type of hemolytic anemia.

Comparison With Human Disease

Microscopic features and tumorigenesis of hepatic angiosarcoma from rats and mice induced by VC resembles closely that which develops in man exposed to VC, thorotrast, and arsenic.[8] In rodents, primary angiosarcomas can also be found in spleen, mesentery, subcutaneous tissue, and other sites. In man, the liver is the only organ affected. Furthermore, VC induces a variety of tumors other than hepatic angiosarcoma in rodents, including hepatocellular carcinoma, skin tumors, and osteochondroma,[7,9] bronchioloalveolar and mammary gland tumors [4,7] in mice, and malignant lymphomas [4,7] in mice and rats. In man, hepatocellular carcinoma is the only tumor besides hepatic angiosarcoma suspected to be associated with VC exposure.[10]

Usefulness of the Model

Vinyl chloride is able to induce hepatic angiosarcoma in many species of laboratory animals. The model can be used to set up guidelines for the tolerance level of VC for workers in PVC plants. The model also provides an opportunity to study the possible adverse effects on the hepatocytes, including tumor induction, caused by the dysfunction of the other com-

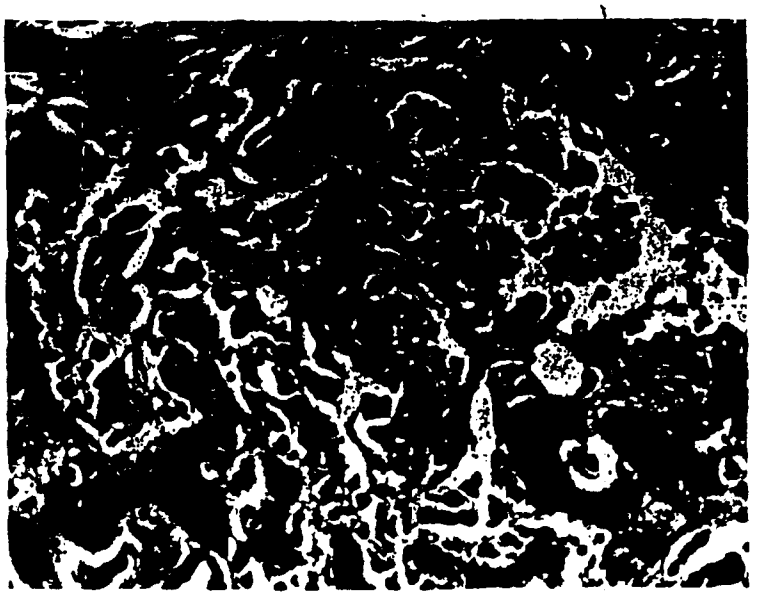

Figure 1—Photomicrograph of liver from a mouse exposed to 1000 ppm VC. Note the early development of angiosarcoma in dilated sinusoids. (H&E, ×940)

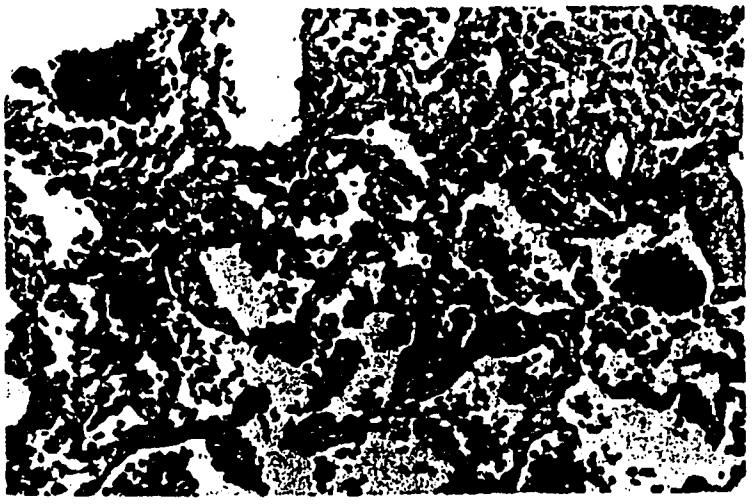

Figure 2—Photomicrograph of liver from a rat exposed to 1000 ppm VC. Note the irregular vascular structures of angiosarcoma. (H&E, ×320)

ponents of liver. Similar to other experimental tumors, this model will add another reproducible system to the study of the tumorigenesis, treatment, and prevention of neoplasms in man.

Availability

Vinyl chloride is commercially available from many chemical manufacturers. All small laboratory animals are susceptible to VC in terms of hepatic angiosarcoma induction. Angiosarcomas can easily be induced in CD-1 mice or CD rats exposed to 250–1000 ppm VC in the air for 6 hr/day, 5 days/wk, within 6–12 months.

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Patterns of Finger Capillary Abnormalities in Connective Tissue Disease by "Wide-Field" Microscopy

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The fingers of 75 patients with connective tissue disorders were examined by "wide-field" capillary microscopy. Four diagnostic groups were included in this study: rheumatoid arthritis—28, scleroderma—22, dermatomyositis—8, and systemic lupus erythematosus—17. On the basis of different patterns of elementary microvascular abnormalities and their distribution, 3 distinct groups were recognized among these patients: 1) increased visibility of nailfold subpapillary plexus in rheumatoid arthritis, 2) massive capillary dilatation in scleroderma-dermatomyositis, and 3) focal loss of capillaries and prominence of subpapillary vessels with "punched-out" lesions in systemic lupus erythematosus.

Skin lesions are prominent features of rheumatic or connective tissue diseases. A variety of clinically recognizable cutaneous changes have been described, most of which include alterations at the microcirculatory level (1, 2). Many investigators have observed these changes microscopically *in vivo* (3–19). By this method of intravital microscopy several microvascular anomalies have been described: viz., a) dilatation of capillary loops; b) dilatation of subpapillary venules; c) distortion of the normal architecture of microvessels in the form of excessive elongation, tortuosity, or sacculation; d) loss of capillaries; and f) extravasation of red cells. Such elementary microvascular changes are not specific, having been reported in several different connective tissue diseases, as well as other disorders.

The small field of observation has made it difficult to demonstrate characteristic patterns of these elementary lesions. To compare one disease group to another or to normal controls, it has been necessary to count and/or to measure the anomalous microvessels in one or several fields of observation (19).

In the present study 4 groups of patients with connective tissue disorders have been examined by the "wide-field" method (20) in an attempt to define patterns of microvascular pathology in these four disease categories. Three separable patterns of microvascular abnormalities can be recognized.

MATERIALS AND METHODS

Subjects

Seventy-five patients attending the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital with the following connective tissue disorders were studied: rheumatoid arthritis—28, scleroderma—22, dermatomyositis—8, and systemic lupus erythematosus—17. They ranged in age from 5 to 73 and included 63 women (25 RA, 16 SD, 6 DM, 16 SLE) and 12 men (3 RA, 6 SD, 2 DM, and 1 SLE).

All patients with RA could be classified as classical or definite by the revised American Rheumatism Association Criteria (21). The diagnosis of SD was made by the definite presence of hidebound skin, often with biopsy confirmation. The diagnosis of DM was made by the presence of myositis, requiring muscle weakness, elevated serum levels of muscle-

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Table 1. Selected Clinical Parameters of Patients Studied

	Rheumatoid arthritis (N = 28)	Scleroderma (N = 22)	Dermatomyositis (N = 8)	Systemic lupus erythematosus (N = 17)
Age, mean, years	48	46	34	31
(range)	(15-72)	(16-62)	(5-73)	(9-68)
Sex, female/male	25/3	16/6	6/2	16/1
Duration, years				
from symptom onset	12.5	5.3	1.3	7.1
from diagnosis	11.0	2.3	1.1	4.6
Raynaud's phenomenon	—	19/22	0/8	—
Therapy				
Aspirin	21	5	0	4
Gold	17	0	0	0
Steroids	3	4	8	14
Immunosuppressive*	1	0	2	3
Vasoactive†	0	9	0	0

*Azathioprine (3), cyclophosphamide (1), or methotrexate (2).
†Guanethidine

derived enzymes and abnormal electromyographic findings, and the characteristic dermatitis of the hands or face. All patients with SLE fulfilled at least 4 of the 14 criteria recently suggested for that diagnosis (22).

Patients with features of more than one rheumatic syndrome ("mixed" or "overlap" syndromes) were excluded from the study. Because of the clinical variability of the syndrome, patients with polymyositis were also excluded from the present study.

Patients with well-established diagnoses were examined at all stages of their disease without selection. Selected clinical features of these patients are shown in Table 1.

Methods

The nailfold area of all 10 fingers was examined in each patient under a wide-field stereo microscope (magnification ×12) after placing a drop of immersion oil on the skin to facilitate visualization of the vessels. The light source for observations was a low-voltage lamp which delivered no appreciable heat to the skin area under study. Other parts of the fingers (finger pads, dorsum of fingers from distal to proximal phalanges) were then inspected for any visible vascular changes which were examined as above. The extent of the visible subpapillary plexus in the nailfolds was measured according to a previously described scoring system (20), which scores each finger from 0 to 4 based on the size of the area where the subpapillary plexus can be seen. The total score for 10 fingers is the plexus visualization score (PVS) of the individual. No attempt was made to measure individual capillary loops.

Although the capillaries are not exactly alike in any given area of the skin, the field of observation under a microscope is rather uniform in normal subjects (Figures 1A and 2A). With the wide field of observation, it was possible to observe patterns of dilated or deformed microvessels and to contrast them with surrounding normal capillaries, the appearance of which was known from previous experience. Extravasation of red blood cells was also recorded.

Observations were supplemented by photographs taken with a single-lens reflex camera fitted with a macrophotographic lens and bellows using the light of an electronic flash, as previously described (20).

RESULTS

Rheumatoid Arthritis

A large number of patients with rheumatoid arthritis (RA) had an extensive visible subpapillary plexus in the nailfold (Figure 1B). Twenty-two of 28 patients (79%) had a PVS of more than 10 points, a value observed in approximately 3% to 6% of healthy adults (23, 24). The fingers of rheumatoid patients were also unusually pale, requiring 2 to 4 times less photographic exposure than

Fig 1. (A) Typical nailfold capillary pattern of a normal adult. (B) Nailfold capillary pattern of a patient with rheumatoid arthritis. Note the subpapillary plexus (SP) and the "shadows" of deeper and larger venules (V). (C) Nailfold capillary pattern of a patient with dermatomyositis. Note the presence of large dilated capillary loops near the edge of the nailfold in both c and d. (D) Nailfold capillary pattern of a patient with scleroderma.

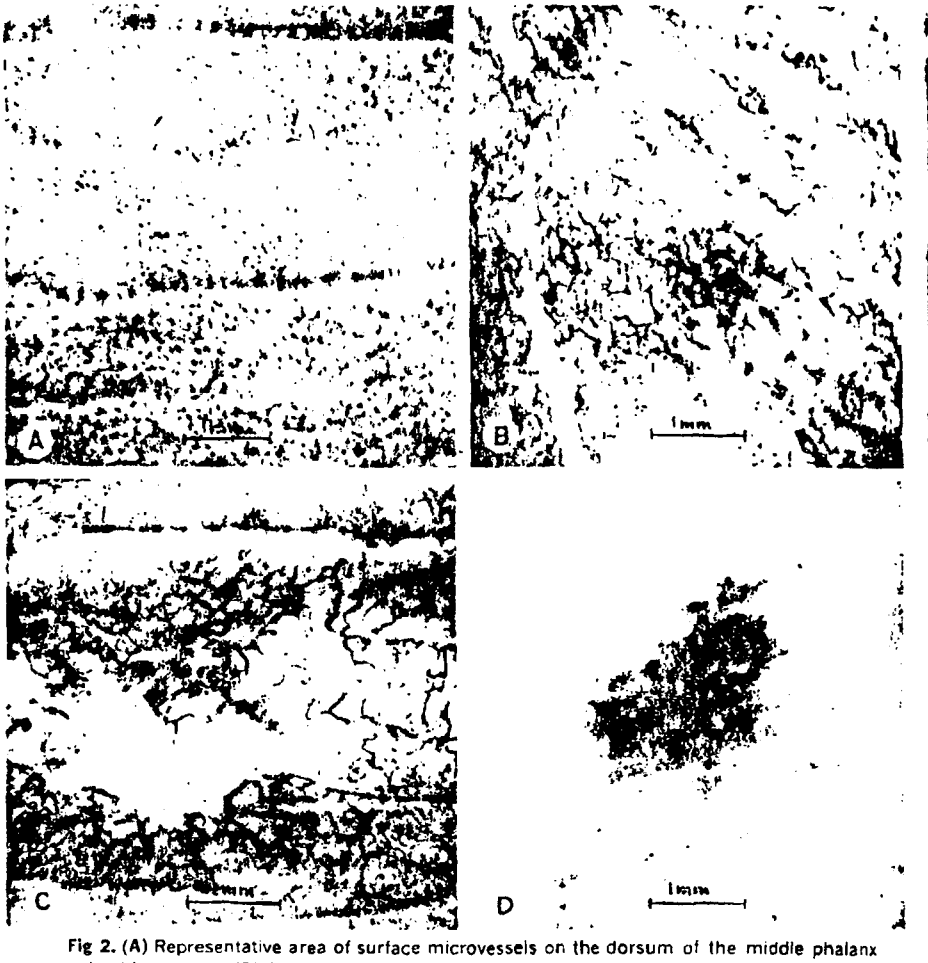

Fig 2. (A) Representative area of surface microvessels on the dorsum of the middle phalanx in a healthy subject. (B) Prominent subpapillary plexus and scarcity of capillary loops on the dorsum of the middle phalanx of a patient with SLE. (C) A "window" lesion on the dorsal finger skin of a patient with chronic SLE showing clinically visible erythematous lesions on the finger. (D) A cluster of large dilated capillaries on the side of the proximal phalanx in a patient with scleroderma.

those of normal subjects. Capillary hemorrhages were noted in 6 patients. No grossly visible vascular alterations were observed clinically on other parts of the fingers in RA subjects; these areas were therefore not systematically studied under the microscope. The examination was difficult in many patients due to hand deformities. Prominent visible venules on the palmar side of the fingers were noted in 5 patients; palmar erythema, in 2 patients. Thus

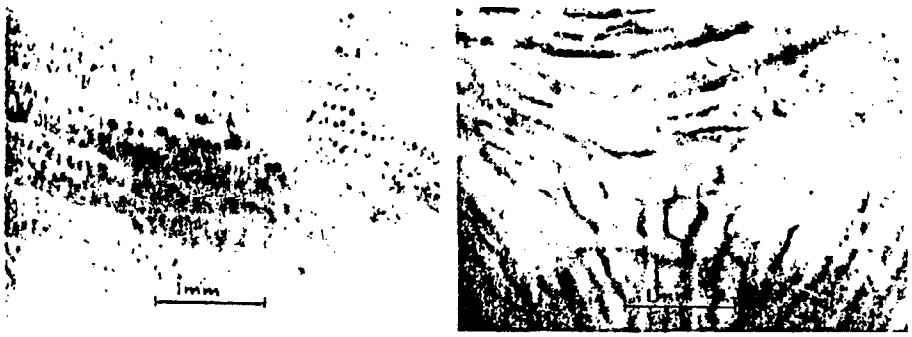

Fig 3. (Left) Finger pad of a patient with scleroderma. Note the cluster of dilated capillaries contrasting with the normal capillaries visible along the finger ridges. (Right) Finger pad of a patient with SLE. Note the absence of capillaries and prominence of the subpapillary plexus compared to usual ridge pattern visible in lower right corner.

the predominant pattern in rheumatoid subjects was an extensive visible subpapillary plexus of the nailfold.

Scleroderma and Dermatomyositis

These two diagnostic categories presented many similarities and no major differences in their vascular patterns, and the results are therefore described together.

In both groups the most striking finding was the presence of enormously dilated capillaries contrasting with the surrounding capillary loops. These giant capillaries were especially well seen in the nailfold (Figures 1C and 1D), but clusters of dilated capillary loops occurred also on more proximal parts of the fingers (Figure 2D) and on finger pads (Figure 3, Left). The dilated capillaries were purple and frequently seen on a background of pale skin. Groups of moderately dilated capillaries were also seen, but these were not characteristic and could also be observed in the systemic lupus erythematosus group. Small avascular scarred areas surrounded by a circle of dilated capillaries were noted in the knuckles. The subpapillary plexus in the nailfold was seen less frequently here than in the rheumatoid group and was of a different character, being associated with obviously atrophic skin. Subpapillary plexus was occasionally also observed in proximal finger skin where telangiectatic spots were visible to the naked eye. Capillary hemorrhages were frequent in both categories of patients and were especially numerous in the 2 patients with acute dermatomyositis who died a few days after examination.

There were only 3 patients in the scleroderma (SD) group who had not reported having Raynaud's attacks. Microvascular patterns in these patients were similar to those of other patients with SD and all 3 had large dilated capillary loops in their nailfolds. None of the 8 patients with dermatomyositis (DM) had experienced Raynaud's phenomenon.

Systemic Lupus Erythematosus

This group of patients had the widest variety of microvascular anomalies and the greatest variation from patient to patient. This could be due to the fact that, whereas patients with SD or DM all had clinically recognizable involvement of the fingers, only 6 patients with systemic lupus erythematosus (SLE) had obvious lesions on the fingers. In these 6 patients, areas of loss of capillaries with prominent subpapillary plexus were the major findings (Fig-

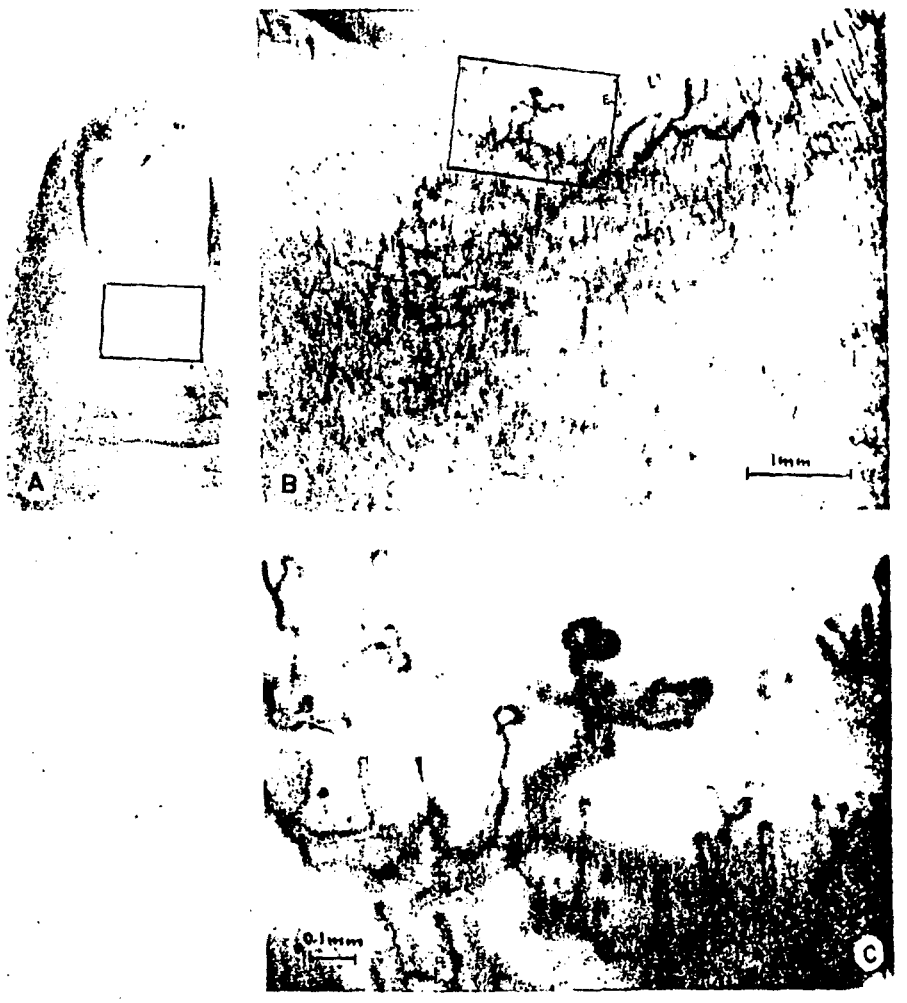

Fig 4. (A) Finger of a patient with scleroderma. Some capillaries can be seen by the naked eye. (The skin is slightly oily from preceding microscopic examination and makes such observations easier). (B) Nailfold vascular pattern of the same finger with the wide-field technique. (Area within frame of Figure 4A at higher magnification.) (C) A portion of the same nailfold. (Area within frame of Figure 4B at higher magnification using Leitz microscope with 6.5× Ultropak objective).

ures 2B, 3Right, 5A, and 5B). In 1 acute case these lesions were diffuse and accompanied by extravasation of red cells and a yellowish exudate (Figure 2B).

The more chronic lesions gave the impression of "punched out windows" in the skin through which deeper vascular layers could be seen. The center of the lesion was almost avascular, with

Fig 5. (A) Nailfold vascular pattern of one of the fingers of the patient shown in Figure 2C. Note the relative similarity of the portion of the nailfold (**upper right**) and the more proximal "window" lesion (**lower left**) seen in this picture. **(B)** Nailfold of a patient in an acute phase of SLE. Note the prominent subpapillary plexus and groups of sharply defined dark capillaries contrasting with pale areas where no capillaries can be seen (leukopenia, hypocomplementemia, erythrocyte sedimentation rate (ESR) = 150). (C) The same nailfold of the patient shown in Figure 5B 4 months later, after partial control of SLE activity (ESR 75, normal white blood cell count (WBC) and serum complement). **(D)** *The same nailfold* 8 months after Figure 5B. The patient was in remission (normal ESR, WBC, and serum complement).

Table 2. Microvascular Observations

	Rheumatoid arthritis (N = 28)	Scleroderma (N = 22)	Dermatomyositis (N = 8)	Systemic lupus erythematosus (N = 17)
Nailfold				
Large dilated capillaries	0/28*	17/22	7/8	1/17
PVS > 10 (visible subpapillary plexus)	22/28	6/18	1/7	7/15
Other parts of finger				
Clusters of dilated capillaries	0/28	12/16	6/8	0/17
Generalized visible subpapillary plexus	—	0/14	0/7	4/14
"Window" lesions	—	0/22	0/8	5/17
Capillary hemorrhages	6/28	16/22	6/8	14/17

*Number of subjects with given abnormality in the numerator, total subjects examined for that feature in the denominator. The denominator varies for certain observations due to pigmentation in dark-skinned subjects which interfered with visualization of capillaries to a varying degree at each anatomic site.

rare subpapillary vessels (Figure 2C). Even in patients whose hands showed no clinical lesions a subpapillary plexus was frequently seen (if not in the nailfold then on the proximal finger skin). There were also groups of small, sharply defined capillaries—darker than the surrounding capillaries—that gave the skin a mottled microscopic appearance. It was difficult to estimate capillary dilatation here because only the tips of vessels were visible. Occasionally, clusters of definite enlarged capillaries were seen in addition to the mottling. Large nailfold loops were observed in only one patient, but unlike scleroderma, the giant loops were associated with an adjacent "punched out" lesion (Figure 5A). Capillary hemorrhages were frequently observed in patients with SLE, with or without obvious skin involvement. A tabulation of elementary lesions seen in the three groups is shown in Table 2.

DISCUSSION

Most investigations of capillary microscopy of human skin have been performed on the nailfold because it is technically the easiest area to examine. Although skin lesions in connective tissue disease may involve the nailfold areas, few authors describe the gross appearance of nailfold skin or mention gross lesions when reporting capillary microscopy.

Other investigators have studied individual skin lesions in various areas of the body and compared these with the skin of healthy people (3, 5–7, 9, 10, 13). Because of the small field of observation, however, only a few elementary microvascular lesions are visible in previous illustrations. (For example, giant capillaries have been reported in SD and DM by many previous workers who have scanned the nailfold area, but their illustrations include only a very limited number of capillaries.) Although in 1953 Gilje advocated capillary microscopy as a "biopsy" of living tissue and proposed the technique as an aid in differential diagnosis, it has not found broad clinical application. From the present work it is proposed that disease entities are characterized by specific patterns of elementary lesions rather than any specificity of the elementary lesions themselves; moreover, wide-field microscopy is essential for the observation of patterns of elementary lesions rather than the individual lesions separately. (Elementary lesions seen in such patterns are similar to and in agreement with those reported earlier by smaller field techniques.) Although use of loupes and low-power microscopes has been recommended earlier, capillary microscopy has

generally been restricted to small field photographs and drawings.

In the present study the wide-field method has allowed observations of patterns of vascular abnormalities and comparisons with surrounding "normal" skin, allowing one to relate the microscopic examination to clinically visible vascular lesions. Skin areas with erythematous spots barely detectable grossly can be characterized by wide-field microscopy and smaller areas can easily be selected from the wide-field patterns for study at higher magnification. The sequence of photographs from the same skin area shown in Figure 4 illustrates this point. The wide-field technique also facilitates sequential observations of microvascular lesions (Figures 5B, 5C, and 5D) because of the ease in relocating the skin area and lesion. This work also demonstrates that the examination of other parts of the finger adds microvascular information in these disorders without the additional equipment needed for examination of other parts of the body.

The results of this investigation (Table 2) show overlap of elementary microvascular lesions in all 4 groups of disorders. On the basis of the pattern and distribution of these elements on the finger, 3 distinct patterns can be recognized:

1. The presence of visible subpapillary plexus in the nailfold without gross dilatation or deformation of capillary loops was characteristic of the RA group. This pattern is not specific for RA and can be found in other conditions such as rheumatic fever, chronic rheumatic heart disease, arteriosclerosis (12), and schizophrenia (24).

2. Large dilated capillary loops in the nailfold and on finger pads or proximal phalanges were characteristic of scleroderma-dermatomyositis patients and may have diagnostic value.

3. In the SLE group, the most characteristic finding was the loss of capillaries and prominence of subpapillary vessels in finger skin where the subpapillary plexus is normally not visible. This phenomenon was most pronounced in discoid lesions but was also present to a lesser degree where only ill-defined lesions were seen.

The circle of horizontally-oriented capillaries surrounding a small avascular area found especially on knuckles in SD, DM, and sometimes in SLE apparently represents a phase in wound healing (25).

Since this study was designed to explore and define patterns of microvascular lesions in various connective tissue disorders, the specificity of the proposed characteristic patterns should be verified by another study in which microvascular patterns are examined "blindly" from photographs.

ACKNOWLEDGMENTS

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Submitted for publicatio 2, 1973.

BEIDLEMAN

Capillary Abnormalities in Polyvinyl Chloride Production Workers

Examination by In Vivo Microscopy

Hildegard R. Maricq, MD; Maurice N. Johnson, MD;
Charles L. Whetstone, MD; E. Carwile LeRoy, MD

• Examination by wide-field capillary microscopy of the hands of 152 workers in vinyl chloride (VC) polymerization plants demonstrated scattered, scleroderma-like microvascular abnormalities in 21 workers and isolated capillary abnormalities in 27, as compared with only three isolated abnormalities in 50 manual workers not exposed to vinyl chloride. Thirteen of 17 VC workers with objective evidence of VC-associated abnormalities (angiosarcoma or fibrosis of liver, acroosteolysis, or scleroderma-like skin lesions) were observed to have microvascular abnormalities.

If prospective studies confirm the implications of this study, capillary microscopy may become a useful mass-screening procedure in the early detection and prevention of VC-associated disease.

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INITIAL reports of human disease in which vinyl chloride (VC) has been implicated as pathogenetic were limited to descriptions of such peripheral changes as distal phalangeal resorption, ie, acroosteolysis (AOL).[1-3] Recently, a systemic effect of VC has been suggested by an unusual pattern of hepatic portal, intralobular, and capsular fibrosis; angiosarcoma of the liver; and pulmonary functional abnormalities suggesting early pulmonary fibrosis, all of which occur more frequently among workers employed in polyvinyl chloride production plants than among the general population.[4-10] Furthermore, animal experiments have demonstrated that VC can produce a variety of tumors in rodents.[11,12]

There are several similarities between VC disease and scleroderma (systemic sclerosis): Raynaud phenomenon is prominent among the symptoms associated with AOL[2,5,6,9,13]; localized scleroderma-like lesions have been reported in some patients with AOL[5,13]; and, in both VC-associated disease and scleroderma, small-blood-vessel lesions and interstitial fibrosis are thought to be important in pathogenesis.[10,12,14]

In scleroderma, changes in small blood vessels of the skin have been observed in vivo by capillary microscopy.[15,16] Using the technique of wide-field capillary microscopy, distinctive abnormal capillary patterns in the fingers of patients with Raynaud syndrome and scleroderma have been demonstrated.[16] Furthermore, a positive correlation is noted between the severity of capillary pattern abnormalities and multisystem involvement in these connective-tissue disorders.[17] The possibility that VC-associated disease might be characterized by microvascular abnormalities similar to those observed in scleroderma led to the present study of VC workers, using techniques of wide-field capillary microscopy. The specific questions of this study were the following: (1) Do symptomatic VC workers have finger-skin-capillary abnormalities? (2) Are the abnormalities similar to or different from those seen in patients with idiopathic scleroderma and Raynaud syndrome? (3) Are microvascular abnormalities related to the type of VC-associated abnormality (peripheral vs systemic), especially liver abnormalities? (4) Are microvascular abnormalities related to the nature and duration of VC-exposure? (5) Are microvascular abnormalities found in a control group of manual workers not exposed to VC?

SUBJECTS

VC Workers Selected by Symptoms (n = 44).—The 44 subjects in this group gave a history of symptoms and signs possibly related to VC exposure. They were 31- to 57-year-old men (mean age, 42 years) who had worked in the polyvinyl chloride industry for 5 to 28 years (mean, 15 years). A history of past reactor-cleaning experience (ranging from 0.2 to 8 years; mean, 2.5 years) was present in 35 of 44 workers. Table 1 shows the complaints and the pathologic findings in group 1.

VC Workers Selected by Question-

From the Division of Rheumatology and Immunology, Department of Medicine, Medical University of South Carolina, Charleston (Drs Maricq and LeRoy); The B. F. Goodrich Company, Akron, Ohio (Dr Johnson); and Continental Oil Company, Ponca City, Okla (Dr Whetstone).

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Two of the seven patients with major bleeding episodes in the intermittent therapy group were switched from intermittent to continuous treatment. These two patients were selected because of ease of observing bleeding and because of need for further treatment with anticoagulants (both had pulmonary emboli). One patient had gross hematuria and one had extensive surgical wound bleeding. Both bleeding episodes were allowed to stop, with the return of the aPTT to normal prior to instituting continuous treatment, and neither patient was given a bolus of heparin prior to continuous heparin therapy. Neither patient demonstrated any recurrence of bleeding while on continuous treatment. Two patients from the continuous therapy group were changed to intermittent therapy; one because of difficulty maintaining an intravenous line and one because the attending physician thought that continuous therapy was inadvisable. The latter patient had no evidence of bleeding in the seven days on continuous therapy, but four days after starting intermittent therapy, heavy surgical wound bleeding ensued and treatment had to be stopped. For statistical purposes, this patient is counted as a nonbleeder on continuous treatment.

Pulmonary Emboli

Three patients were suspected of having pulmonary emboli while on heparin therapy because of symptoms of either acute dyspnea and chest pain, pleuritic chest pain, or hemoptysis. Repeat lung scans in the latter two patients did not show evidence of emboli (one patient on intermittent treatment and one on continuous treatment). The first patient had a pulmonary embolus proved by pulmonary arteriogram while on continuous heparin therapy for disseminated intravascular clotting. This patient had an aPTT of 49 seconds on the day before the pulmonary embolus, and an aPTT taken at the onset of symptoms was shortened to 22 seconds. No change in the mode of continuous heparin therapy had been instituted in the interim. This suggests had developed warfarin resistance and embolism.

COMMENT

In this study, patients receiving intermittent heparin therapy had significantly more major bleeding episodes (33%) than patients receiving continuous therapy (0%). This agrees with the findings of Salzman et al[2] who reported 1% major occurrences of bleeding in patients on continuous heparin treatment vs 8% in the intermittent group. However, Mant et al[5] found no difference in bleeding episodes between continuous and intermittently treated patients. In their study, continuously treated patients received a significantly greater daily heparin dose than the intermittent group, whereas in the present study and in the one by Salzman et al,[2] intermittently treated patients received significantly higher doses. The discrepancy between the results of these studies may be explainable on this basis: Mant et al[5] had 12.5% major hemorrhages in their continuous therapy group receiving 34,151 units of heparin daily compared to no major hemorrhages in our patients receiving 24,000 units of heparin daily.

It seems probable that the transiently high blood levels of heparin achieved with intermittent therapy predisposes these patients to hemorrhage.

Minor bleeding episodes in the present study were also more frequent, but not significantly so, in the intermittent therapy group (48%) of patients than in the continuous therapy group (30%). In our study, one patient (2%) died of hemorrhage, which is similar to the mortality (2%) in the report by Salzman et al.[2] As in a previous report,[3] our bleeders were significantly older than nonbleeders, but we could find no female preponderance.

The use of antiplatelet drugs probably is unwise in patients on full-dose heparin treatment, and aspirin may have been a predisposing factor in the two patients who bled heavily when receiving intermittent treatment. The number of patients receiving aspirin in the continuous therapy group was similar to that in the intermittent.

It is difficult to indict the concomitant use of warfarin and heparin in a bleeding diathesis since the majority of patients in this study were given both drugs simultaneously at some time, but only two patients bled while receiving both drugs. Basu et al[3] suggests that keeping the aPTT greater than 1½ times the control will prevent pulmonary emboli in patients on continuous therapy. Five of the 162 patients in that study had a clinically evident recurrence, generally when the aPTT was less than 1½ times the control for more than two to five days. We could not define a lower limit of heparin dosage to prevent recurrent thromboembolism since only one patient had proved pulmonary emboli while on treatment, nor could we define an upper limit for continuous treatment since no patients in the group bled excessively.

Because the question arises of whether patients who are bleeding while receiving intermittent treatment can continue to receive anticoagulants, two patients with severe bleeding episodes on intermittent treatment were switched to continuous treatment and in neither case did bleeding resume. This indicates that some patients who bleed while receiving intermittent treatment need not have their anticoagulant permanently discontinued if the heparin can be given by continuous infusion.

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naire (n=108).—These subjects were selected on the basis of their answers to a questionnaire used in the polyvinyl chloride production plant to detect early AOL. All available subjects who had given one or more affirmative answers to the questionnaire (Q+) were examined, a total of 66 subjects. Seventy-one subjects were examined from a random selection of those who had given negative answers to all questions (Q−). Data on 29 of the 137 subjects examined were excluded because of unreliable observations caused by skin injuries or deep pigmentation, leaving 108 employees (50 Q+ and 58 Q−) in this group.

Control Manual Workers (n=50).—The possibility that repeated minor injuries associated with manual labor might contribute to the abnormal findings seen in VC workers led to the examination of workers from companies making neither VC nor polyvinyl chloride and hospital employees working as carpenters, truck drivers, and maintenance men, a total of 50 workers. All were men and ranged in age from 19 to 62 years (mean age, 43 years).

METHODS

Wide-field Capillary Microscopy.—The technique of wide-field capillary microscopy as previously reported[16] consists of the observation of selected skin sites under immersion oil, using a stereo wide-field microscope at magnification ×12. Wide-field microscopy permits the detection of microvascular patterns, such as the presence and distribution of dilated or deformed microvessels and the absence of capillary loops, without attempting to measure absolute vessel dimensions. The technique is rapid and suitable for mass screening.

Twelve standard sites were examined in each subject: four nailfolds and two each of the following sites: dorsum of the distal phalanx other than the nailfold, dorsum of the middle phalanx, dorsum of the proximal interphalangeal joint, and the finger pad.

Photography of Microvessels.—The wide-field photographic technique as previously described[16] was used to photograph at least one finger of each subject. All photographs were coded for blind comparison of capillary findings in VC workers and control subjects.

Clinical and Occupational Data.—All subjects were interviewed as to length of employment, job assignment, general state of health, presence or absence of Raynaud phenomenon, and smoking and drinking habits. A limited physical examination was performed. All data were coded for later analysis and were unknown to the capillary observer and photographer.

Table 1.—Clinical Findings and Complaints of Group 1 Vinyl Chloride Workers*

	No. of Subjects Examined	No. of Subjects With Capillary Changes†
Severe AOL with Raynaud phenomenon	6	4
Scleroderma-like skin lesions with Raynaud phenomenon	5	4
Liver abnormalities‡	6	5
Subtotal	17	13
Healed AOL with Raynaud phenomenon	6	0
Healed AOL without Raynaud phenomenon	1	0
Raynaud phenomenon alone	9	3
Nonspecific skin rashes without Raynaud phenomenon	7	1
Miscellaneous complaints	4	0
Subtotal	27	4
Total	44	17

*AOL indicates acroosteolysis.
†Of 17 subjects with abnormal capillaries, ten had scattered changes (8/13 and 2/4 of the two groups above) and seven had isolated changes.
‡Two patients with angiosarcoma of the liver and one each with (1) portal fibrosis and enlarged spleen, (2) portal fibrosis with early cirrhosis and fatty metamorphosis, (3) thrombosis of the splenic vein, mild liver damage, and reticuloendothelial cell hyperplasia, (4) fatty metamorphosis with focal capsular and subcapsular fibrosis and splenomegaly with vascular congestion.

Table 2.—Comparison of Capillary Observations Between VC Workers and Controls*

	Employees of VC Industry	Manual Workers Not Exposed to VC
Scattered capillary abnormalities	11	0
Abnormal capillaries present, but few in number	27	3
No capillary abnormalities seen	70	47
Total	108	50

*VC indicates vinyl chloride. Difference in the prevalence of capillary abnormalities between VC workers and controls is statistically significant ($\chi^2 = 15.50$, $P<.001$).

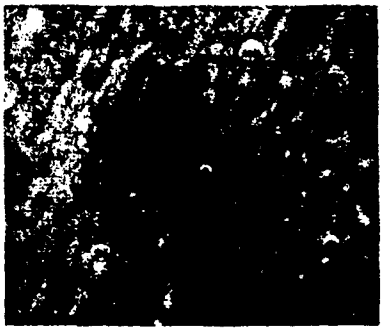
RESULTS

Group 1 (VC Workers Selected by Symptoms).—Examination of group 1 subjects showed scattered capillary abnormalities similar to those found in scleroderma (Fig 1 and 2). Definitely dilated capillary loops were seen in the nailfold and in other finger sites, contrasting with the surrounding normal vasculature. Pale avascular areas were frequently observed. The capillary abnormalities were usually more discrete and less numerous than those seen in most patients with scleroderma. They were seen more frequently in subjects with severe AOL, liver abnormalities (as defined in Table 1) and scleroderma-like lesions (13/17) than in those with healed AOL, nonspecific skin rashes, or Raynaud phenomenon alone (4/27; $\chi^2 = 14.21$, $P < .001$). Moreover, 5 of 6 subjects with liver abnormalities verified by biopsy specimens had microvascular abnormalities (Table 1).

In a few subjects, lesions resembling mini versions of clinically visible papular skin lesions were observed microscopically (Fig 3, left). In two subjects with active AOL, capillary hemorrhages were observed under the proximal nail plate in addition to the common type of "splinter hemorrhages" under the distal nail plate (Fig 3, center).

Group 2 (VC Workers Selected by Questionnaire).—Dilated capillaries were observed in 38 of 108 group 2 workers; both the number and distribution of dilated capillaries differed from those observed in most scleroderma patients. Many group 2 workers had isolated, extremely dilated capillaries (Fig 3, right and Table 2). No correlation was found between the length of time employed as a reactor-cleaner (or the length of time with the company) and the capillary abnormalities. Office and supervisory personnel who had never been produc-

Fig 1.—*Top left*, Wide-field view of nailfold area in normal subject. Bottom left, Nailfold capillaries of patient with scleroderma. Bottom right, Nailfold capillary pattern in worker of polyvinyl chloride production plant. Note similarity with pattern seen in scleroderma patients.



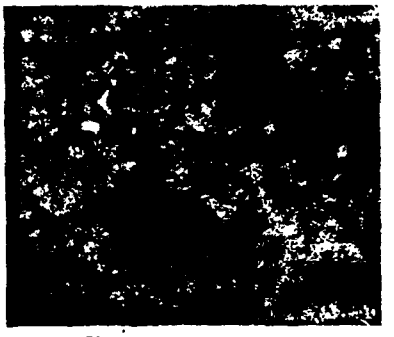
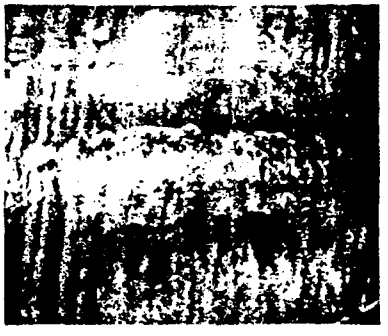


Fig 2.—Left, Dorsum of middle phalanx in normal subject. Center, Cluster of dilated capillaries in same anatomical site in patient with scleroderma. Right, Vinyl chloride worker with pattern similar to that seen in scleroderma.

Fig 3.—Left, Miniplaque on dorsum of distal phalanx in VC worker with acroosteolysis (AOL). Center, Capillary hemorrhages under both distal and proximal nail plate in a vinyl chloride (VC) worker with AOL. Right, Single extremely dilated capillary in nailfold of VC worker with liver abnormalities.



tion workers had fewer microvascular abnormalities (3/22) than production workers (35/86, $\chi^2 = 4.49$, $P < .05$); however, production workers showed no differences in capillary abnormalities according to job category. There was no significant difference ($\chi^2_{df} = 1.32$, $P > .5$) between employees who had been classified as Q+ or Q− on the basis of the questionnaire. It was noted, however, that there was little or no correlation between the responses regarding Raynaud phenomenon obtained from the questionnaire and those obtained from the interview at the time of examination. Physical examination disclosed two workers with AOL (one "active" and one "healing" by roentgenographic examination) and papular skin lesions, and ten workers with hepatomegaly (4 cm or more below the right costal margin). The subject with active AOL showed scattered capillary changes, the subject with healing AOL had a "normal" capillary examination. There was no correlation between hepatomegaly and capillary findings.

Comparison of VC Workers With Control Subjects.—Capillary abnormalities in group 2 VC workers were significantly more frequent than in the control manual workers examined ($\chi^2 = 15.50$, $P < .001$) (Table 2).

Analysis of Photographic Data.—Three hundred seventeen unidentified photographs of VC workers were mixed with 136 photographs of control subjects and 130 photographs of patients with scleroderma and related diseases; all photographs were rated for the presence of capillary abnormalities. The results demonstrated that significantly more VC workers (39/152) than control subjects (3/50) had capillary abnormalities ($\chi^2 = 7.69$, $P < .01$). Although objective, for technical reasons, photographs are slightly less sensitive than direct observations in detecting capillary abnormalities.

COMMENT

The results of this study demonstrate that capillary abnormalities seen in the finger skin by in vivo microscopy are present in VC workers more frequently than in control subjects. These capillary abnormalities are not exclusively related to peripheral pathological conditions such as AOL, scleroderma-like lesions of hands, and Raynaud phenomenon, but are also found in subjects with liver abnormalities (as defined in Table 1) and in subjects who have papular lesions elsewhere on the body. Microscopic papular or fibrotic lesions can also be observed in VC workers. In 33% of the subjects who show any capillary changes, the pattern appears similar to the microvascular changes seen in scleroderma; in the remaining majority, changes consist of a few dilated capillaries only; very few control subjects show even these minimal capillary abnormalities. Since microvascular changes occur more frequently than overt VC-associated pathologic conditions in VC workers, they may represent an early manifestation of the VC effect.

More work is needed to determine the nature and evolution of such microvascular abnormalities, their relationship to VC-associated disease (as opposed to a transient reaction to VC exposure), and their relationship to the subject's susceptibility to VC disease. Further studies are also needed to compare the capillary changes reported here with those seen in small blood vessels and endothelia of animals exposed to VC.[11] Capillary microscopy might then be used as a reproducible, noninvasive technique to monitor VC workers and to identify VC-associated peripheral and visceral disease in susceptible individuals at an early and possibly preventable stage.

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Drug Addiction Among Physicians

The Virginia Experience

Robert C. Green, Jr, MD; George J. Carroll, MD; William D. Buxton, MD

• **Drug addiction among physicians appears to be an occupational hazard, with chronic pain, depression, and the easy availability of drugs major factors leading to addiction. In this study of 46 cases of physician addicts handled by the Virginia State Board of Medicine, meperidine hydrochloride (Demerol) was the most frequent addictive agent. The Virginia disciplinary and therapeutic plan for addicted physicians was effective in successfully rehabilitating and returning to medical practice 72% of the 46 physician addicts reported to the board from 1949 to 1974.**

(*JAMA* 236:1372-1375, 1976)

AS DRUG addiction in the general population has increased, awareness of addiction as an occupational hazard among physicians has also developed. In 1957, the US Commissioner of Narcotics estimated that 1 of 100 physicians was addicted, as compared with 1 of 3,000 in the general population.[1] In New York state alone in a 25-year period, 0.5% of all licensed physicians were reported as addicts to the Bureau of Narcotics Control.[2] A 1969 estimate[3] of 300 new physician addicts per year is probably now too conservative.

This communication analyzes 46 cases of drug-addicted physicians handled by the Virginia State Board of Medicine from 1949 to 1974, with emphasis on background, personality disorder, and probable causes of addiction. We also discuss the board's disciplinary, therapeutic, and rehabilitative scheme.

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Our study may seem small, but the board believes these 46 cases are merely the "tip of the iceberg." Too often, the problem is handled locally, by revoking hospital privileges or by inducing the addicted physician to move to another state, without attempts at treatment and rehabilitation.

RESULTS OF VIRGINIA STUDY

From 1949 to 1960, less than one case per year was reported to the board. Since 1960, the yearly incidence increased to a peak of five cases in 1968, but has gradually declined since. One case was recorded in 1974, although two additional cases were reported but not included in this study (Fig 1).

The majority of initial reports on physician addicts in Virginia came from state and federal narcotics inspectors, with state mental institutions and hospitals the next largest source. Their distribution is as follows: state and federal narcotics investigators, 26; institutions, 10; individuals, 5; patients, 2; other state boards, 2; and Medical Society of Virginia, 1.

Regrettably, legislation passed in the 1974 Virginia Assembly no longer makes involuntary commitment of drug-addicted physicians a mandatory reportable offense to the state board. Reporting of these cases is now up to the treating psychiatrist's judgment, so there may well be fewer cases coming to the board's attention.

Physicians' ages in initial reports to the board ranged from 27 to 73 years, with a median of 45 years. It appears significant that a stair-step progression developed, peaking in the 45- to 49-year group with 13 of the 46 cases. The incidence in the older age group was markedly lower.

The number of years in practice relates directly to the frequency of addiction (Fig 2). Median duration of practice was 18 years, followed by a gradual decline in incidence of drug dependency.

Information on duration of addiction prior to its being reported was available in only 32 cases, but 16 were reported within a year of onset. It is noteworthy that some addicted physicians could go on for 10, 16, and, in one instance, 18 years, without being apprehended.

Median age of onset of addiction was 43 years, with seven physicians in the 45- to 49-year group (Fig 3). Eight physicians' ages ranged from 22 to 34 years, and two were in the

symptoms of influenza. Both women had primary influenzal pneumonia which was at its peak about the 7th day from the first signs of infection. One woman died on the 12th day, and influenza virus was recovered from lung, liver, spleen, tracheal swabs, plasma, and buffy coat. It now seems clear that viræmia can be present in certain cases of influenza from the day before the first respiratory symptoms[40] throughout the first week of illness.[39] The virus can be present and multiply in lung, liver, and skeletal muscle of ferret and mouse in the absence of light microscopical evidence of viral infection.[36 31 41]

A myopathy can complicate influenza infection on about the 7th day.[12–15] Enzymatic evidence of a myopathic lesion has been identified in some patients with Reye's syndrome.[42]

We believe that certain cases of primary influenzal pneumonia in previously healthy persons, the myopathy associated with influenza, and Reye's syndrome, all of which become evident about the 7th day after the initial respiratory signs of influenza infection, may represent "7th day" dissemination of influenza virus to the affected tissues. This hypothesis has the merit that it can be tested with simple tools already at hand.

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PORTAL HYPERTENSION IN VINYL-CHLORIDE PRODUCTION WORKERS

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Summary Portal hypertension was seen in seven patients who had been involved in the production of vinyl-chloride monomer for 4–15 years. Four presented with bleeding œsophageal varices. In liver biopsy specimens non-cirrhotic portal fibrosis was inconspicuous and was seen more clearly on wedge rather than needle biopsy specimens. The intrahepatic venous pattern was hardly disturbed. Three patients have done well following shunt surgery. In one patient an angiosarcoma developed, but fibrosis was a more common lesion and was probably not pre-malignant.

Introduction

FORTY-SEVEN cases of hepatic angiosarcoma have so far been identified in workers exposed to vinyl chloride,[1] but there have also been reports of acro-osteolysis, scleroderma-like changes, Raynaud's phenomenon,[2–4] pulmonary ventilation disturbances, fibrosis of the portal tracts,[5] portal hypertension, splenomegaly, and thrombocytopenia.[6] In the past 9 years we have seen seven vinyl-chloride-monomer (V.C.M.) production workers with portal hypertension in a large chemical plant producing over 100 000 tonnes of poly-vinyl-chloride annually, and we describe our findings.

Case-reports

The seven patients were aged 36–57 years at the time of presentation, having been exposed to V.C.M. gas for 4–15 years, for at least part of the time as a polymer reaction cleaner (table I). Four had a hæmatemesis from œsophageal varices and two of these were known to have had splenomegaly and thrombocytopenia beforehand because of prior investigation for abdominal pain and a skin rash respectively. One patient, who had had a 15-year duodenal ulcer history, had an enlarged fibrotic liver at laparotomy and large collateral veins, and later died from an hepatic angiosarcoma. Two subjects were found to have thrombocytopenia during a survey, and this was later shown to be due to hypersplenism associated with portal hypertension. Six of the patients had hepatomegaly and four a palpable spleen.

Liver function tests showed non-specific and relatively minor abnormalities (table II). One patient was minimally jaundiced, another had a raised serum-alkaline-phosphatase,

PROFESSOR PARTIN AND OTHERS: REFERENCES—*continued*

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TABLE I—DETAILS OF 7 MALE V.C.M. WORKERS WITH PORTAL HYPERTENSION

Case no.	Age (yr)	Exposure (yr)	Presentation	Liver (cm)	Spleen (cm)
1	43	15	Abdominal pain. Hæmatemesis.	4	4
2	48	6	Skin rash. Hæmatemesis.	4	2
3	39	6	Hæmatemesis.	4	4
4	36	4	Duodenal ulcer.	4	0
5	57	13	Hæmatemesis.	6	0
6	32	8	Thrombocytopenia on survey.	0	0
7	43	10	Thrombocytopenia on survey.	4	4

and two had raised transaminase levels. Four out of six tested had an abnormal bromsulphalein retention. Plasma-protein values and serum electrophoresis were normal except for one low albumin level.

Low platelet-counts of 39 000–80 000/mm³ were present in five out of six subjects. Tests for antinuclear factor, hepatitis-associated antigen, and mitochondrial and smooth-muscle antibodies were negative or very weakly positive, except for case 2, who had smooth-muscle antibodies detected in his serum in a titre of 1/64. No patient gave a history of hepatitis, contact with hepatotoxins such as arsenic, or ingestion of known hepatotoxic drugs, but one (case 5) drank to excess, consuming a bottle of wine a day.

Intrasplenic pressure in cases 2, 5, 6, and 7 was raised at 30, 25, 30, and 24 mm Hg. The wedged hepatic vein pressure in cases 6 and 7 was slightly increased at 12 and 14 mm Hg respectively. Splenic venography was performed in every case, demonstrating the presence of a patent portal vein and œsophageal varices. Despite this, the intrasplenic venous pattern appeared normal or only very slightly abnormal in four patients.

At laparotomy the liver appeared finely nodular and cirrhotic, but biopsy revealed a non-cirrhotic fibrosis in four patients (cases 1, 3, 5, and 7) and slight fibrosis in cases 2 and 6. There was loss of portal-vein branches in cases 2 and 7. The degree of fibrosis in most patients was relatively minor, mainly involving the portal tracts only. In case 2 a needle biopsy specimen was normal, and the fibrosis was detected only when a wedge biopsy specimen was taken during a portacaval shunt. Case 4, who later developed an angiosarcoma, showed a remarkable focal severe dilatation of the sinusoids, and has been separately reported.[7]

Of the four patients who bled from œsophageal varices, one died two months after an œsophageal transection from hepatic failure and *Escherichia coli* septicæmia. The other three (cases 2, 3, and 5), have done extremely well after end to side portacaval shunts, surviving so far for 3, 6, and 8 years respectively. All are on an unrestricted protein intake and show no signs of encephalopathy apart from a trace of hepatic fetor. Two have returned to full-time work. Case 6 has recently had a small variceal hæmatemesis and is being considered for surgery.

Discussion

Much publicity followed the discovery of angiosarcoma in workers exposed to vinyl chloride.[8,9] but it is our experience that non-cirrhotic portal fibrosis with associated portal hypertension is a commoner lesion. The fibrosis has not yet proved malignant in that six of our cases have been followed for 3–8 years with no deterioration in liver function; all have been removed from contact with V.C.M. The three workers who have undergone a portacaval shunt have fared particularly well, suggesting that hepatic parenchymal function is preserved. The apparently normal intrahepatic vascular pattern seen in some of our patients on splenic venography, and the high intrasplenic pressure with a slightly raised wedged hepatic vein pressure, indicating a predominantly presinusoidal obstruction to portal flow, are similar to the findings in non-cirrhotic portal fibrosis seen in India[10] and in chronic arsenic ingestion.[11]

Hepatic fibrosis was also observed in V.C.M. workers by Lange et al.,[5] who described capsular and portal fibrosis, œsophageal varices, splenomegaly, and thrombocytopenia in addition to thickening of the collagen bundles of skin. Thomas et al.[12] found in 14 workers exposed to V.C.M. an inconspicuous intralobular and a conspicuous capsular fibrosis in tumour-free portions of liver, with an increase in perisinusoidal reticulin. It has been postulated that a metabolic product of vinyl chloride binds to plasma-protein producing a conformational change within the protein molecule.[13] This acts as an antigen that stimulates antibody production. Antibody and antigen then form a soluble complex, which is cryoprecipitable and can activate complement, and can result in platelet aggregation, thrombocytopenia, and vascular occlusion. The vascular occlusion would stimulate new collagen biosynthesis.[14] While this mechanism may be responsible for hepatic fibrosis and portal hypertension, we believe that the thrombocytopenia is a manifestation of the hypersplenism, as reduced hæmoglobin and white cell levels have been seen in some instances, and there is no improvement on removal from contact with V.C.M.

It has been suggested that one of the results of prolonged exposure to V.C.M. may be splenomegaly due to stimulation of splenic cells with a consequent increase in splenic and hepatic blood-flow.[12] Portal hypertension may result from an inability to accommodate this increased hepatic blood-flow because distension of portal vein branches is prevented by portal fibrosis, and that of the sinusoids by capsular and subcapsular fibrosis. A similar mechanism may also operate in Banti's syndrome. Further investigations to determine the contribu-

TABLE II—BIOCHEMICAL AND HAEMATOLOGICAL DATA IN V.C.M. WORKERS WITH PORTAL HYPERTENSION

Case no.	Bilirubin (μmol/l)	Alkaline phosphatase (i.u./l)	Aspartate transaminase (i.u./l)	Albumin (g/l)	Globulin (g/l)	B.S.P. (%)	Hb (g/dl)	W.B.C. (/mm³)	Platelets (/mm³)
1	44	30	1	38	24	..	12·2	6900	
2	17	44	10	36	17	15	10·0	12 000	56 000
3	17	15	38	39	20	21	17·3	4700	80 000
4	17	35	64	38	30	17	14·0	6000	170 000
5	17	115	64	24	31	5	12·0	8300	54 000
6	18	31	20	37	32	7	15·4	6100	61 000
7	11	32	..	34	34	19	13·7	4700	39 000
Normal range	17	15–45	15–32	35–50	15–45	5 (45 min)			

W.B.C. = White blood cell count B.S.P. = bromosulphalein.

tion of splenic blood-flow to the portal hypertension are planned.

With improvements in technology, workers should no longer be exposed to levels of V.C.M. gas above 5 p.p.m. and it is hoped that no new cases of portal hypertension and hepatic fibrosis will be seen. However, we are left with the problem of how to screen workers exposed to high concentrations of gas in the past. As hepatic parenchymal function is preserved, tests that rely upon conventional liver function tests have proved disappointing but we have identified two workers with portal hypertension through thrombocytopenia secondary to hypersplenism. However, we believe that conventional tests are not sufficiently sensitive and preliminary work suggests that grey-scale ultrasonography may be more successful in detecting abnormalities.[15]

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PROSTAGLANDINS AND ASPIRIN THERAPY IN BARTTER'S SYNDROME

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Summary A young patient with Bartter's syndrome was treated for three months with 100 mg/kg/day of aspirin to inhibit prostaglandin synthesis. Clinical symptoms resolved and serum-potassium increased from 2·9 to 3·5 mmol/l. Urinary excretion and plasma concentration of prostaglandins E and F were significantly reduced during aspirin therapy. Plasma-renin activity declined from 85 to 20 ng/ml/h (normal 1·5–4 ng/ml/h) and hyperaldosteronism was corrected. These results suggest that overproduction of prostaglandins has a central role in the pathogenesis of Bartter's syndrome.

Introduction

Bartter's syndrome is an uncommon disorder characterised by hypokalæmia, hyperreninæmia, hyperaldosteronism, juxtaglomerular hyperplasia, normotension, and resistance to the pressor effects of angiotensin II. The pathogenesis is unknown and treatment has been largely unsatisfactory. It has been suggested[1–3] that overproduction of renal prostaglandins is of pathophysiological importance. This study further examines the role of prostaglandins in the pathophysiology of Bartter's syndrome and describes the successful use of aspirin in the treatment of a patient with this disorder.

Case-report

A 22-month-old, 7 kg girl was referred to Walter Reed Army Medical Center following a 13-month history of muscle weakness, growth retardation, polyuria, and polydipsia. Development was normal. Hypokalæmia (serum-potassium 2·4 mmol/l) was noted three months before admission and persisted despite 40 mmol/day of potassium-chloride supplements. Physical examination was unremarkable except that height and weight were both below the first percentile. Blood-pressure was 90/60. A diagnosis of Bartter's syndrome was based on persistent hypokalæmia with inappropriate urinary potassium losses, raised plasma-renin activity, increased urinary aldosterone excretion, and exclusion of other known causes of hypokalæmia. In response to 5 mmol/day sodium restriction the patient excreted less than 1 mmol/day in the urine. After 5 days of oral salt loading, plasma-renin activity was suppressed from 74 to 47 ng/ml/h. A percutaneous renal biopsy specimen disclosed vascular thickening with juxtaglomerular transformation of arteriole cells, increased number of immature glomeruli, and atrophy of macula-densa cells. The renin

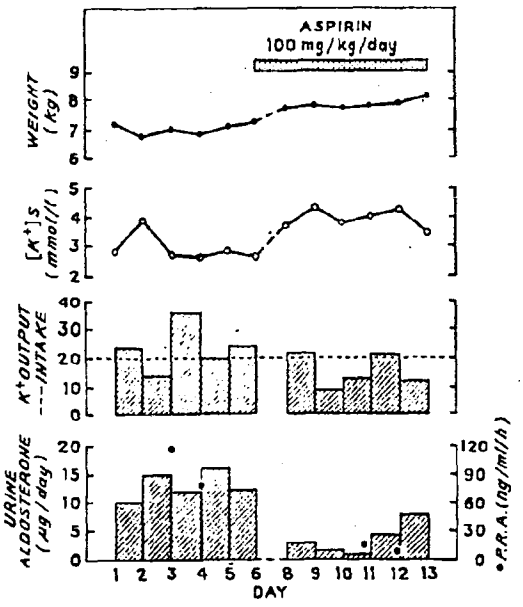

Fig. 1—Effect of aspirin on plasma-renin activity, aldosterone excretion, and potassium balance.

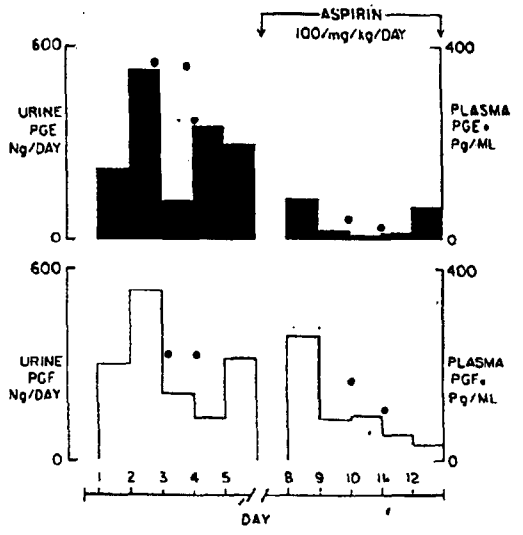

Fig. 2—Acute effect of aspirin on urine and plasma prostaglandins.

activity of a juxtaglomerular apparatus isolated from the biopsy specimen[4] was 181 ng/h. This value is three times greater than any other human specimen we have analysed (unpublished observations).

Materials and Methods

During the balance study (figs. 1 and 2) the patient received a metabolic diet containing 20 mmol of sodium and potassium per day. Intake and urinary excretion of electrolytes were monitored daily; fæcal losses were not measured. Blood-samples were obtained at 8 A.M. with the patient supine. Plasma-renin activity and urinary aldosterone were measured by radioimmunoassay. Immunoreactive prostaglandins of the E-type (P.G.E) and F-type (P.G.F) were determined in plasma and urine as previously described.[5] All prostaglandin analyses were done in duplicate and the variation between paired samples did not exceed 10%.

Results

The results shown in figs. 1 and 2 demonstrate the acute effects of aspirin. During control observations (days 1–5) the patient's weight remained constant and she was hypokalæmic and in negative potassium balance. Plasma-renin activity was markedly raised at 119 and 72 ng/ml/h (normal 1·5–4 ng/ml/h). Urinary aldosterone excretion, uncorrected for body surface area, averaged 14 μg/day (normal adult <20 μg/day). Daily urinary excretion of P.G.E and P.G.F averaged 225 and 252 ng respectively. Plasma-P.G.E ranged from 224 to 363 pg/ml and plasma-P.G.F was 218 pg/ml. The patient was given 100 mg/kg/day of aspirin in divided doses beginning on day 6. The same measurements were taken for five consecutive days beginning on day 8. During this interval the serum-salicylate was 15–20 mg/dl. After beginning aspirin, as shown on the right hand portion of fig. 1, the patient gained weight, potassium balance became positive, and serum-potassium increased. Corresponding to the 0·6 kg weight-gain was a combined cumulative positive balance for sodium and potassium of 75 mmol. Plasma-renin activity decreased to 22 and 10 ng/ml/h and urinary aldosterone excretion was reduced by approximately 50%. The effects of aspirin on plasma and urine prostaglandins are shown in fig. 2. Plasma and urine P.G.E were reduced by approximately 90% whereas plasma and urine P.G.F declined to 50% of control values.

After completing this phase of the study, the patient was discharged from the hospital and maintained on the same dose of aspirin. The same parameters were followed at weekly intervals for 10 wk and these results are presented in the accompanying table. During this time the patient continued to gain weight. Muscle weakness and polyuria resolved. Appetite and activity level increased. Serum-potassium averaged 3·6 mmol/l on aspirin, as compared to 2·9 mol/l during control observations. Plasma-renin activity continued to be suppressed, although not completely into the normal range. Urinary aldosterone excretion remained significantly below pretreatment values throughout the period of observation. In response to aspirin, mean urinary P.G.E excretion declined from 225 to 25 ng/day and P.G.F decreased from 252 to 99 ng/day. Plasma-P.G.E declined from 293 to 23 pg/ml and plasma-P.G.F was reduced from 218 to 110 pg/ml.

After ten weeks of treatment, aspirin was temporarily discontinued for four days. This resulted in a 0·5 kg weight-loss, negative potassium balance, and a fall in serum-potassium to 2·6 mol/l. Plasma-renin activity increased to 85 ng/ml/h and urinary aldosterone increased two fold. Urinary excretion of P.G.E and P.G.F returned to pretreatment values as did plasma-P.G.F. In contrast, plasma-P.G.E increased only slightly. At the time of writing the patient has been on aspirin for 3 months and serum-potassium is 3·5 mmol/l without potassium supplements.

Discussion

Fichman et al.[3] reported the first successful use of indomethacin in a patient with Bartter's syndrome. In their patient indomethacin caused a fall in plasma-aldosterone, plasma-renin activity, and prostaglandin A2. Vascular insensitivity to angiotensin II was reversed and potassium balance improved. Bartter et al.[1] reported increased urinary excretion of P.G.E_2 and P.G.F_2 in 5 patients with this disorder. When these subjects were

DATA FROM PATIENT DURING CONTROL PERIOD AND CHRONIC ASPIRIN THERAPY

	Weight (kg)	Serum-potassium (mmol/l)	Plasma-renin activity (ng/ml/m)	Urine-aldosterone (ug/day)	Serum-salicylate (mg/dl)	Urine-P.G.E (ng/day)	Urine-P.G.F (ng/day)	Plasma-P.G.E (pg/ml)	Plasma-P.G.F (pg/ml)
Control period Mean ±(S.E.M.)	7·0±0·2	2·9±0·1	83±13	14±2	0	225±56	252±59	293±69	218±21
Aspirin-therapy period Mean ±(S.E.M.)	8·0±0·1	3·6±0·1	20±5	6±2	15·8±1·5	25±4	99±11	23±5	110±12
P	<0·01	<0·01	<0·01	<0·01	..	<0·01	>0·05	<0·01	<0·01

After five control observations the patient was given 100 mg/kg/day of aspirin. Observations were then made at weekly intervals for 10 wk.

treated with inhibitors of prostaglandin synthetase, serum-potassium rose and both plasma-renin activity and urinary aldosterone excretion decreased. Verbeckmoes et al.[2] also noted a favourable therapeutic response to indomethacin but prostaglandin measurements were not reported in their subject. The present study provides additional evidence supporting a central role for prostaglandins in the pathogenesis of Bartter's syndrome. In this patient aspirin therapy was associated with a significant fall in urine and plasma prostaglandins, reduction of renin and aldosterone production, and correction of hypokalæmia. It seems unlikely that these results reflect some action of aspirin other than inhibition of prostaglandin synthetase. The changes in urine and plasma prostaglandins were clear cut and aspirin does not usually cause significant alterations in sodium or potassium metabolism.

The stimulus for increased prostaglandin production and the mechanism relating prostaglandins to other aspects of this disorder are not understood. Factors which are known to affect renal prostaglandin production include renal nerve stimulation, catecholamines, bradykinin, angiotensin, ischæmia, and the state of sodium balance. The normal pulse-rate, blood-pressure, and vascular resistance[6] in these patients, does not support overactivity of the adrenergic nervous system as the stimulus for increased prostaglandin production.

Vinci et al.[7] reported a decrease in plasma-bradykinin and urine-kallikrein in several patients with Bartter's syndrome after inhibition of prostaglandin synthetase. This suggests that kinins do not stimulate the overproduction of prostaglandins in Bartter's syndrome, but rather are secondary to increased prostaglandin synthesis. Similarly, in both our patient and those reported by Bartter et al.[1] and Fichman et al.,[3] inhibition of prostaglandin synthetase resulted in a fall in plasma-renin activity suggesting that this disturbance is also secondary to overproduction of prostaglandins.

Renal sodium handling in Bartter's syndrome is a controversial issue. Despite the fact that many of these patients conserve sodium normally, several workers have proposed primary defects in sodium absorption in the proximal ascending limb or distal portions of the nephron.[8–12] Many of these proposed defects in sodium transport may have been secondary to prolonged potassium depletion.[13] In two reports of patients with this disorder[7 14] renal sodium absorption was normal following correction of potassium deficiency. Our patients showed normal renal sodium conservation and displayed no evidence of extracellular volume contraction. This argues against negative sodium balance as a stimulus for increased prostaglandin production.

Irrespective of the stimulus for increased synthesis, several observations suggest that prostaglandins have an important role in the vascular resistance to angiotensin II. This vascular defect is an order of magnitude greater in these patients than in any other disorder and is usually not corrected by expansion of the extracellular volume or replacement of potassium deficiency.[12 14] Several investigators have presented evidence in animals[15 16] and humans[17] showing that prostaglandins are important modulators of the vasoconstrictor response to angiotensin II. Verberckmoes et al.[2] and Fichman et al.[3] have reported reversal of angiotensin II insensitivity in Bartter's syndrome after treatment with indomethacin.

Before the use of prostaglandin inhibitors, the therapeutic focus in Bartter's syndrome was correction of hypokalæmia. Therapy with renin inhibitors, aldosterone antagonists, and potassium supplements alone or in combination have been unsuccessful in maintaining normokalæmia for a prolonged period. In our patient treatment with aspirin alone resulted in resolution of clinical symptoms and correction of hypokalæmia. There were no adverse side-effects. These observations are particularly relevant to the treatment of Bartter's syndrome in pædiatric patients where the safe use of indomethacin and other inhibitors of prostaglandin synthetase has not been established.

We are grateful to Dr Sheldon Sommers for evaluating the renal biopsy and to Ms C. Armstrong for performing the prostaglandin assays.

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TOTAL HIP REPLACEMENT IN RENAL TRANSPLANT RECIPIENTS WITH ASEPTIC NECROSIS OF THE FEMORAL HEAD

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Summary Aseptic necrosis of the femoral head is a complication of renal transplantation which seriously delays the rehabilitation of the patient despite otherwise successful transplantation. Of 187 renal allograft recipients, 8 underwent 14 total hip replacements. The most severe postoperative problem was an easily reducible dislocation. All patients were relieved of their severe preoperative pains and all were greatly improved in mobility and strength. Renal function was not altered in any patient. It is therefore suggested that total hip replacement be recommended in such cases at

BEIDLEMAN

Original Contributions

Hepatic Disease Among Workers at a Vinyl Chloride Polymerization Plant

Henry Falk, MD; John L. Creech, Jr., MD; Clark W. Heath, Jr., MD; Maurice N. Johnson, MD; Marcus M. Key, MD

• Eleven cases of hepatic disease, including seven cases of hepatic angiosarcoma, have been identified to date among men employed at one vinyl chloride polymerization plant. The earliest diagnosis was made in April 1964. The two most recent cases, both angiosarcoma, were diagnosed in February 1974 as a result of systematic medical screening for liver abnormalities among workers at the plant. Ages at diagnosis have ranged from 36 to 58 years for the seven patients with angiosarcoma and from 28 to 56 years for the four patients with nonmalignant disease; durations of employment before diagnosis have ranged from 12 to 28 years and from 5 to 29 years. All 11 persons had worked in close and continuous contact with various phases of the vinyl chloride polymerization process. Review of pathologic material suggests the presence in both tumor and nontumor cases of portal fibrosis and atypical sinusoidal lining cells. A direct causal relationship between exposure to vinyl chloride monomer and pathologic findings is postulated.

(*JAMA* 230:59-63, 1974)

CREECH and Johnson[1] recently reported the occurrence of three cases of angiosarcoma of the liver among workers at a polyvinyl chloride (PVC) production plant in Louisville. Because this tumor is extraordinarily rare (only about 25 cases are estimated to occur each year in the entire United States), the existence of such cases in this particular setting

See also p 64.

strongly suggests a causal relationship to some phase of the PVC production process. Recent animal studies being conducted in Italy support the concept that exposure to vinyl chloride monomer (VCM) may be the mechanism involved (C. Maltoni et al, unpublished data).

Beginning in late January 1974, intensive efforts have been devoted to clinical and epidemiologic studies of past and present workers at the Louisville plant, as well as at other PVC production plants elsewhere, in order to define more precisely the extent of health risks among vinyl chloride workers. This report summarizes findings to date with respect to both malignant and nonmalignant hepatic disease among workers at the Louisville plant, particular emphasis being given to epidemiologic features.

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BACKGROUND

The production of PVC by polymerization of VCM began in Germany about 40 years ago, with production in the United States starting about five years later. The industry grew rapidly after World War II, and growth has continued in recent years at a rate of about 14% per year. Currently in the United States, 14 plants employing about 1,500 workers produce VCM, while 37 plants employing about 5,000 workers polymerize PVC from VCM. Current annual production of PVC in the United States is estimated at approximately 4.4 billion pounds, or 25% of world production.

The B. F. Goodrich PVC polymerization plant in Louisville first began operations in 1942. The number of persons employed at the plant directly in PVC polymerization has steadily increased, reaching a fairly stable level of 250 to 300 workers by the late 1950s. At present, in addition to 271 persons engaged directly in PVC polymerization, about 850 persons are employed at the plant in other activities such as synthetic rubber production, compounding and milling operations, managerial and clerical positions, and maintenance work outside PVC polymerization areas.

Until 1966, VCM as well as PVC was produced at the Louisville plant. Since that time, however, all VCM used at the plant has been shipped by tank car from other facilities. The VCM is unloaded, stored, and then piped into large polymerization reactor vats through an essentially closed system. Each reactor receives a measured amount of VCM as well as appropriate catalysts, stabilizers, emulsifiers, and additives (and other monomeric compounds if copolymers or terpolymers are being produced), and the reaction is carried to the desired end point. Polymerized material is dropped into secondary tanks from which unreacted VCM is recovered and recycled through a closed system; it then enters tertiary tanks from which it is concentrated, dried, and packaged. The end product consists of three different materials: (1) PVC resin (a powder with the texture of refined sugar), (2) PVC paste (a very fine powder with the texture of processed flour), and (3) PVC latex (a stable suspension of PVC in liquid).

For workers in PVC polymerization, the point of greatest probable exposure to VCM occurs after poly-

and Pathologic Findings of Cases of Liver Disease

Physical Findings	Hepatic Work-up*	Pathologic Findings†
1/64-Right upper quadrant tenderness	1/64-Moderate elevation in TB, SGOT	1/64-OLB: slight focal hepatitis 3/64-NLB: unchanged 4/64-PM: hepatic angiosarcoma with metastases
8/67-Epigastric mass and splenomegaly	8/67-Mild elevation in AP, SGOT, LDH Platelet count, 33,000/cu mm Liver scan: large defect, splenomegaly	8/67-OLB: angiosarcoma 1/68-PM: hepatic angiosarcoma with spread to diaphragm and abdominal wall
1/70-No abnormalities 5/70-Hepatosplenomegaly	5/70-Mild elevation in TB, AP, SGOT, LDH Liver scan: large defect Esophogram: varices	5/70-OLB: angiosarcoma No PM
12/63-No abnormalities 5/65-Splenomegaly	5/65-Elevated SGOT Esophagoscopy: varices	5/70-OLB: toxic hepatitis 10/70-NLB: hepatitis, cirrhosis 10/70-OLB: slight hepatitis 3/73-PM: hepatic angiosarcoma
7/73-Hepatosplenomegaly	7/73-Marked elevation in AP Mild elevation in TB, SGOT Liver scan: diffuse disease, hepatosplenomegaly	7/73-NLB: fibrosis OLB: periportal inflammation and fibrosis 12/73-PM: hepatic angiosarcoma spread to duodenum
2/74-No abnormalities	2/74-Mild elevation in LDH Liver scan: possible defect	2/74-OLB: angiosarcoma
2/74-No abnormalities	11/73-Mild elevation in TB, AP Liver scan and angiogram: 4-cm defect	2/74-OLB: angiosarcoma and extensive portal fibrosis, subcapsular fibrosis
11/68-Splenomegaly	10/68-Marked elevation in AP, BSP Mild elevation in SGOT Esophagram: varices	11/68-OLB: portal fibrosis, subcapsular fibrosis
9/71-Hepatosplenomegaly	9/71-Mild elevation in TB, SGOT 10/71-Liver scan: splenomegaly	12/71-OLB: portal fibrosis, subcapsular fibrosis
8/73-Splenomegaly	3/73-Moderate elevation in TB, AP Liver scan: splenomegaly, small liver	9/73-OLB: slight portal fibrosis
9/73-No abnormalities	9/73-Moderate elevation in SGOT Mild elevation in TB, LDH	9/73-OLB: chronic hepatitis with focal fibrosis

*TB indicates total bilirubin; SGOT, serum glutamic oxaloacetic transaminase; AP, alkaline phosphatase; LDH, lactic dehydrogenase; BSP, sulfobromophthalein.
†OLB indicates open-liver biopsy; NLB, needle liver biopsy; PM, postmortem examination.

(case 6), or angiosarcoma with extensive portal fibrosis (case 7). In cases 1 and 2, large hepatic masses were present at initial evaluation. The conditions of three patients (cases 1, 4, and 5) were not diagnosed until autopsy, despite multiple liver biopsies.

Preliminary pathologic review suggests that in all 5 patients for whom nonmalignant hepatic tissue is available (cases 2, 3, 4, 5, and 7), similar nonmalignant hepatic lesions exist, consisting of portal fibrosis, sinusoidal dilation, and atypical sinusoidal lining cells. Conceivably, such lesions may represent a precursor stage in the development of hepatic angiosarcoma.

Nonmalignant Hepatic Disease

Ages at diagnosis for these four patients (cases 8 through 11) range from 28 to 56 years (average age, 46.5). Initial clinical manifestations varied widely: one patient (case 8) had gastrointestinal bleeding; one (case 9) had chest pain and weight loss; and two patients (cases 10 and 11) had unrelated problems. On physical examination, hepatosplenomegaly was present in one patient (case 9), splenomegaly alone in two (cases 8 and 10), with normal findings in one patient (case 11). Three patients underwent splenectomy either for marked splenomegaly (case 9) or as part of splenorenal shunt procedures (cases 8 and 10). Results of liver function tests varied widely and showed no consistent patterns in relation to clinical manifestations.

All four patients were found on liver biopsy to have some degree of hepatic fibrosis. Pathologic review of specimens available from these suggests a close histologic similarity to the manifestations of portal fibrosis and sinusoidal changes described in the cases of angiosarcoma. Three patients (cases 8, 9, and 11), as well as one patient in the angiosarcoma group (case 7), were found at surgery to have a peculiar white, speckled appearance to the surface of the liver, which on pathologic section was seen to reflect diffuse subcapsular fibrosis.

EPIDEMIOLOGIC FINDINGS

The seven men with angiosarcoma had been employed at the plant for 12 to 28 years (average, 18.0), and the four with nonmalignant disease, between 5 and 29 years (average, 20.6)

helpers whose principal job is to clean reactors. As can be seen in Table 3, ten of the 11 patients worked at some time as helpers. While virtually every employee at the plant has worked as a helper before being promoted to more advanced work, the average work duration (ie, time from starting work at the plant to date of diagnosis) was 23.5 years for the five patients who spent nine months or less as helpers and 15.1 years for the six patients who spent 20 months or more. This suggests indirectly a possible relationship between intensity of exposure and latent period for liver disease.

All 11 patients, or members of their immediate families, were individually interviewed regarding past hepatic disease and possible exposure to hepatotoxic agents. None of the patients had a history of hepatitis or of exposure to hepatitis, and none had taken hepatotoxic drugs. Three patients (cases 1, 7, and 9) may have had significant alcohol intake. None, except for the patient in case 7, recalled exposure to possible hepatotoxic chemicals outside the work environment, in particular to either arsenic or thorium dioxide, two chemicals previously implicated as causes of hepatic disease and hepatic angiosarcoma in humans.[4-7] Patient 7 gave a history of exposure to arsenical insecticides on the family farm between the ages of 6 and 15 years; he both mixed and sprayed the insecticides two to three times a year for about three hours on each occasion. In no case was there any history of acro-osteolysis.

COMMENT

Before the report by Creech and Johnson,[1] the only evidence that VCM might be oncogenic came from animal experiments. In 1971, Viola et al[8] published data suggesting oncogenicity of VCM when inhaled by rats at very high doses; tumors of many tissue sites, including lung, bone, and skin were recorded. Preliminary results of a more recent animal study in Italy by Maltoni et al (unpublished data) appear to indicate that angiosarcoma of liver as well as of other tissues can be induced in rats by atmospheric levels of VCM that are not uncommon in the human workplace environment. In light of these observations, it appears likely that exposure to VCM is responsible for the Louisville cases. Further support for this hypothesis is needed, of course, from additional epidemiologic data concerning workers at other PVC and VCM plants. Further toxicologic analyses are also needed to address the possibility that the active oncogenic material may be some metabolite of VCM instead of VCM itself.

Whatever the precise mechanism for oncogenicity and hepatotoxicity of VCM, the Louisville data suggest that relatively high levels of VCM exposure and relatively long intervals since first exposure (20 years or so) are involved. This does not rule out the possibility of less marked health effects at lower doses or at shorter intervals of exposure, but it does for the present focus attention on the immediate problem of assessing the health status of persons exposed in the remote past to high doses. None of the Louisville cases involved men working at the plant less than six years before diagnosis, and it can be safely assumed that levels of VCM exposure during earlier years of PVC production were considerably higher than at present because of less stringent work practice procedures and less attention to minimizing possibilities of VCM exposure in places of work.

In humans, both thorium dioxide and arsenic have previously been reported as causes both of hepatic disease and of angiosarcoma of the liver. In only one case at the Louisville plant (case 7) was there any history of exposure to either of these two materials (arsenic in insecticide spray). Likewise, there is little or no evidence among the Louisville cases that excessive alcohol intake plays any accelerating or potentially cocarcinogenic role or that any direct relationship exists between acro-osteolysis and liver disease.

Various data now suggest that VCM (or some derived metabolite) may produce in addition to angiosarcoma of the liver a nonmalignant hepatic disorder characterized by portal fibrosis and portal hypertension. This is suggested by the fact that such fibrosis was present in at least five of the Louisville tumor cases and was in addition observed in four other Louisville vinyl chloride workers without tumor. Recent observations in Germany,[9] together with earlier reports from Eastern Europe,[10] suggest that hepatic fibrosis and portal hypertension represent an occupational disease not uncommon among vinyl chloride workers. Conceivably, such fibrotic liver disease represents a premalignant state. If this proves to be so, the early detection of such liver disease may be of greater industrial and public health importance than detection of tumor itself, both because hepatic fibrosis may well emerge as a more frequent condition than tumor in vinyl chloride workers, and because it remains a possibility that very early liver abnormalities may be reversible or nonprogressive after workers have been removed from high-risk areas.

Laszlo Makk, MD, Lynn Ogden, MD, Edward Fadell, MD, Anne Richman, MD, Curtis Songster, MD, George Schrodt, MD, Edward Callahan, MD, Jerry Clanton, MD, James Kurfees, MD, Stanley Seipel, MD, and Will Ward, MD, supplied records and pathologic materials.

Louis Thomas, MD, and Hans Popper, MD, reviewed pathologic material.

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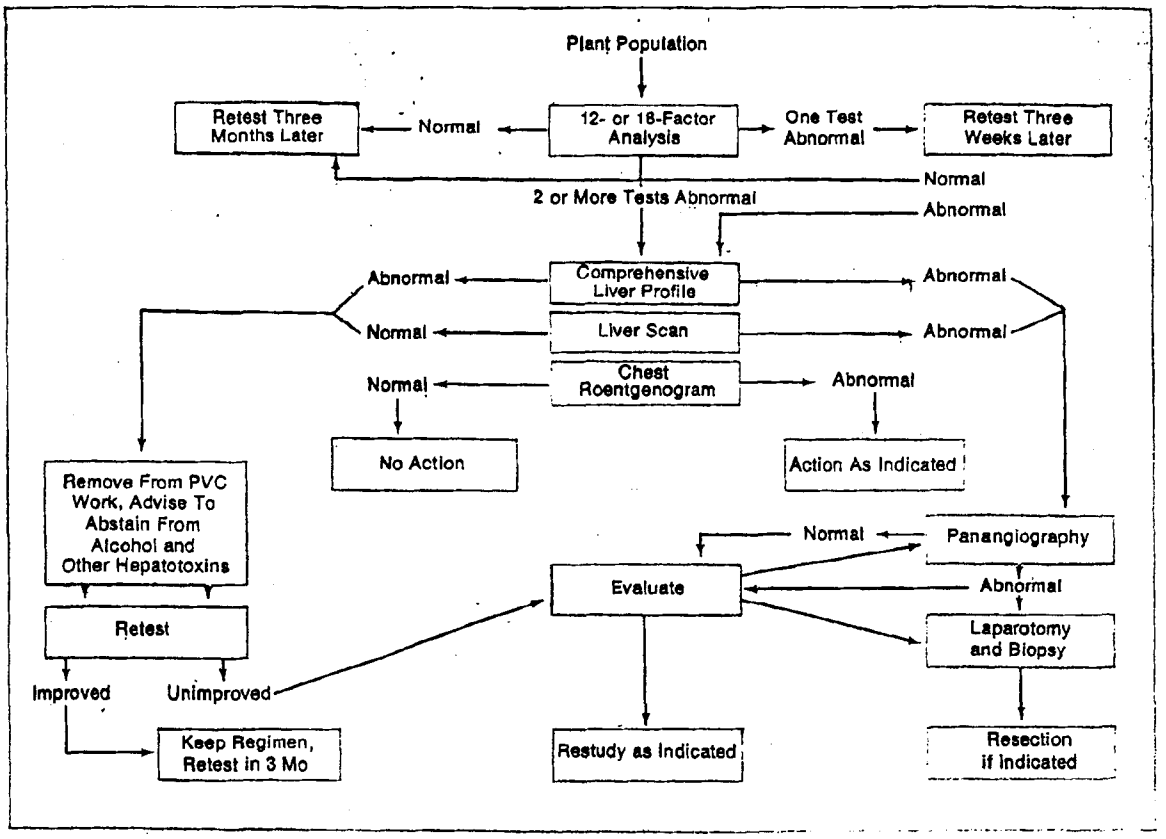

Fig 1.—Liver disease detection protocol.

mality was found, the 12-factor analysis was repeated in three weeks. If the single abnormality presisted, or if two or more liver-related abnormalities were found on the initial laboratory tests, the patient underwent a comprehensive examination consisting of tests for the following values: a 12-factor automated chemical analysis, serum electrophoresis, LDH isoenzymes, fractionated alkaline phosphatase, direct and indirect bilirubin (if total level was elevated), serum glutamic pyruvic transaminase (SGPT), γ-glutamic transpeptidase (GGTP), isocitrate dehydrogenase (ICD), α-fetoprotein (fetoglobulin), and carcinoembryonic antigen (CEA). Also, a complete blood cell count (CBC), platelet count, chest roentgenogram, and liver and spleen scan were done.

Abnormalities found in the initial 12-factor chemical analyses indicating the need for comprehensive profiles were grouped according to the following work areas: (1) PVC production, (2) synthetic rubber production, and (3) all others (including maintenance, shipping, laboratory, and other salaried employees).

Table 1.—Liver Enzyme and Bilirubin Results

Test	No. of Patients Studied	No. (%) With Abnormal Values	Normal Range	Abnormal Range
Alkaline phosphatase, mU (milliunits)/ml	72	35 (49.4)	3-85	87-135
γ-Glutamic transpeptidase, mU/ml	70	31 (44.3)	6-28	29-575
Serum glutamic oxaloacetic transaminase, mU/ml	68	19 (29.8)	4-25	27-559
Bilirubin, mg/100 ml	72	19 (26.4)	0.15-1.0	1.1-2.6
Serum glutamic oxaloacetic transaminase, mU/ml	73	13 (18.0)	12-40	48-150
Isocitrate dehydrogenase, mU/ml	59	9 (18.0)	0-7	8-88
Lactic dehydrogenase, mU/ml	72	8 (11.1)	90-225	237-475

Liver Abnormalities Dictated Hospital Studies

If abnormal scan or other findings suggested serious liver disease or the possibility of angiosarcoma, the pa-

Table 3.—Tissue, Roentgenologic, and Laboratory Examination Correlation

Liver Scan	Level of: Alkaline Phosphate	γ-Glutamic Transpetidase	Serum Glutamic Pyruvic Transaminase	Bilirubin	Serum Glutamic Oxaloacetic Transaminase	Lactic Dehydrogenase	Platelet Count
Filling defect and cirrhosis	↑	↑↑	↑	↑	↑	N↑	↓
Filling defect, both lobes	↑	↑	N	N	↑	↑	↑
Suggestive of cirrhosis	↑	↑	↑	↑	↑	N	N
Suggestive of cirrhosis	↑	↑	↑	N	N	N	N
Suggestive of cirrhosis	↑	↑	N	N	N	N	N
Normal	↑	N	N	N	N	N	N
Normal	↑	N	N	N	N	N	N

factor chemical analysis. Of these, 75 (6.3%) had either two liver-related abnormalities on the initial screening or one such abnormality that persisted. These patients had comprehensive evaluations. One patient had initial screening in the plant, further evaluation and biopsy elsewhere, and then was transferred to our institution for further studies. Results of alkaline phosphatase, GGTP, SGPT, bilirubin, SGOT, ICD, and LDH examinations are listed in Table 1.

Significant Biochemical Findings

Other tests of the comprehensive liver profile yielded the following significant results: moderately elevated total protein values were found in three patients with a similar elevation in γ-globulin content. Serum electrophoresis showed slight to moderate increase in γ-globulin in 11 patients. The β-globulin value was slightly lowered in six patients.

In 32 patients with elevated alkaline phosphatase values, 15 had increased liver fractions. In 37 patients with normal levels of alkaline phosphatase, fractionation was also performed. Thirteen showed relative elevation of liver fractions. Eight of 72 patients (11.1%) showed elevated values for total LDH. Four of these had isoenzyme 4; two, isoenzyme 5; and one, isoenzyme 3 elevation; one had normal isoenzyme proportions.

Of 64 cases with normal LDH values, 44 showed relative elevation in level of isoenzyme 4 (68.7%); two, relative isoenzyme 5; and one, relative isoenzyme 1.

Fetoglobulin determinations gave normal results in all cases. Results of carcinoembryonic antigen radioimmunoassays were normal in all patients, with one marginal result in a patient who smoked. Complete blood cell counts disclosed normal values for hemoglobin, hematocrit, red blood cell (RBC) count, and corpuscular indexes in each patient. Four patients had slight to moderate granulocytosis. One patient showed relative lymphocytosis. Twenty-three patients showed slight, and seven moderate, monocytosis. Three patients had moderately increased eosinophil counts. One patient had thrombocytopenia with a platelet count of 98,000/cu mm, and another had thrombocytosis with a count of 750,000/cu mm (normal range, 140,000 to 440,000). The number of 12-factor abnormalities and comprehensive profiles according to work areas is listed in Table 2.

Radiological Studies

Seventy-four patients had liver scans: 11 of these scans (14.8%) were abnormal, and 2 were borderline. Six patients had filling defects (Fig 2). Three of these and another five patients had irregularities suggestive of cirrhosis. Spleen scans were performed concurrently with liver scans in 71 patients.

Three patients had had splenectomies in the past. Nine patients (12.7%) had enlargement of the spleen to 22 cm (normal spleen size is considered less than 14 cm in length[2]). Splenomegaly with abnormal liver scan was present in three patients.

Hepatic panangiography was performed in seven patients. By panangiography, two of the liver scan filling defects were found to be tumors (Fig 3 and 4). The anomalous position of the gallbladder in one case, and dilatated short gastric and collateral portal veins in another correlated with the defects on the scans. The other angiograms were normal.

Of the six splenic arteriograms, two showed splenomegaly; one of these with multiple splenic aneurysms and the other, intrasplenic arterial strictures. The other four were normal.

Surgical Findings

Exploratory laparotomy was performed in seven patients; in two, angiosarcoma was found. Resection could not be carried out in one patient because of the extent of neoplasm. In the other patient, angiosarcoma was limited to one lobe, but because of portal fibrosis in the other lobe, resection was deferred.

In three patients, varying degrees of portal fibrosis was found on laparotomy. Of the remaining two patients, one had a cholecystectomy, the other an incisional hernia repair. Liver biopsy was performed concomitantly on each. Both of these livers were essentially normal on biopsy and on laboratory testing. Correlation of biopsy, roentgenographic, and laboratory findings is given in Table 3.

VINYL-CHLORIDE-INDUCED LIVER DISEASE

From Idiopathic Portal Hypertension (Banti's Syndrome) to Angiosarcomas

LOUIS B. THOMAS, M.D., HANS POPPER, M.D., PH.D., PAUL D. BERK, M.D., IRVING SELIKOFF, M.D., AND HENRY FALK, M.D.

Abstract Histologic examination of liver tissue (eight autopsy and 18 biopsy specimens) and five spleens from 20 workers with vinyl chloride polymerization showed hepatic angiosarcomas in 15. In addition, a peculiar pattern of progressive portal-tract, inconspicuous intralobular and conspicuous capsular fibrosis was observed in the five workers without angiosarcoma, in all the seven patients with angiosarcoma from whom tumor-free portions of the liver were available, and in two tumor-free biopsies from patients subsequently found to have angiosarcoma. The fibrosis was accompanied by splenomegaly. Hypertrophy and hyperplasia of both hepatocytes and hepatic and splenic mesenchymal cells were also seen. The histologic similarity to chronic inorganic arsenical poisoning, in which angiosarcomas also occur, and to idiopathic portal hypertension (Banti's syndrome) suggests that the latter syndrome at times results from unknown toxic, possibly environmental, chemicals. (N Engl J Med 292:17-22, 1975)

THE development of hepatic angiosarcomas in workers exposed to vinyl chloride gas in the manufacture of polyvinyl chloride has been well documented.[1-3] Histologic study of hepatic and splenic specimens taken from such workers suggests to us, moreover, that other diseases that have not in the past been related to industrial exposure may be involved. Specifically, a case will be made for the concept that splenomegaly with portal hypertension associated with slight hepatic fibrosis, previously designated as Banti's syndrome,[4] may be the result of exposure to known or unknown chemical agents.

HISTORY OF THE VINYL CHLORIDE LIVER INJURY

Polyvinyl chloride, one of the most widely used synthetic plastics, has been manufactured for more than 40 years by polymerization of gaseous vinyl chloride in the United States and in many other countries. Concern about untoward side effects in workers had centered primarily on a disease, acro-osteolysis,[5] which is characterized by Raynaud's syndrome, dermal induration and bone lesions. Two reports[6,7] also dealt with nonspecific alterations of hepatic structure and function, and a lesion designated "chronic epithelial hepatitis" was found in about 25 per cent of the examined workers in Russia.[6] Hepatic abnormalities, which did not attract major attention, became far more important because of the discovery of three cases of angiosarcomas of the liver, an otherwise very rare tumor, in workers in a polyvinyl chloride production plant in this country.[1] The introduction of a surveillance system in this plant detected a total of seven cases of hepatic angiosarcoma.[2,8] Animal experiments confirm the relation between hepatic angiosarcoma and exposure to gaseous vinyl chloride. In 1971, Italian investigators reported that prolonged inhalation of vinyl chloride produced carcinomas of the zymbal gland in rats,[9] and Maltoni[10] subsequently described angiosarcomas of the liver and other organs as well as nephroblastomas in rats exposed to vinyl chloride gas. Recently, angiosarcomas of the liver have been seen in mice after exposure to as little as 50 ppm of vinyl chloride.[11]

When specimens of human angiosarcoma from vinyl chloride polymerization workers were reviewed in the Laboratory of Pathology of the National Cancer Institute, the lesions in areas of the liver not involved by angiosarcoma appeared similar to alterations recently reported in vinyl chloride workers in Germany.[12] In these patients inconspicuous portal and perisinusoidal fibrosis was associated with impressive hepatic capsular fibrosis as seen by peritoneoscopy. Hepatic-function tests revealed variable abnormalities. Clinical manifestations included portal hypertension with splenomegaly, thrombocytopenia and bleeding esophageal varices. This German report focused our interest on the appearance of the liver not only in patients with hepatic angiosarcomas but also in other workers with hepatic fibrosis exposed to vinyl chloride who had been diagnosed as having cirrhosis because of portal hypertension, variceal hemorrhage and splenomegaly.

By coincidence, there have been several recent reports of portal hypertension without obvious cause in patients with psoriasis who had received an inorganic arsenic preparation, Fowler's solution, for prolonged periods.[13-15] These observations were interesting in view of earlier reports from Germany and France of hepatic angiosarcomas developing in vintners exposed to insecticides containing inorganic arsenic.[16] Roth's report included 47 workers with chronic arsenic intoxication. Of these, four had hepatic carcinomas, five had sarcomas (angiosarcomas), and 13 of 27 autopsied by him had unusual types of cirrhosis.[16]

MATERIAL STUDIED

The observations recorded here are based on the study of hepatic tissues obtained from 20 workers who had industrial exposure to vinyl chloride for prolonged periods, usually exceeding two years and extending up to 18 years. The tissues were derived from 15 patients with angiosarcoma and five with hepatic fibrosis but without angiosarcoma. The majority of these specimens were initially obtained, studied, and in some cases reported by others,[1-3] but all were submitted to the Laboratory of Pathology, Na-

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pressed the surrounding parenchyma, and in one tumor, anaplastic sarcoma cells invaded portal-vein branches.

Focal dilatation of sinusoids with proliferation and enlargement of the sinusoidal cells associated with perisinusoidal fibrosis may represent an early stage in the evolution of angiosarcoma. This lesion was noted in six of the seven available tumor-free liver specimens from patients with angiosarcoma, and in the two biopsies taken before angiosarcoma was detected. It was also observed in two patients in whom angiosarcoma has not developed, and in whom, accordingly, careful follow-up observation is indicated. In the patients with angiosarcoma, some of the sinusoidal lining cells had enlarged, atypical nuclei and bulky cytoplasm that was PAS negative in contrast to many of the lining cells in the uninvolved parenchyma (Fig. 2). There were gradual transitions between the areas of focal dilatation of sinusoids to multiple, microscopic-sized angiosarcomas with a sinusoidal pattern.

In three patients other organs besides the liver were involved by angiosarcoma: the duodenum in one, the lung in a second, and the lung, heart, kidney and lymph nodes in a third patient.

Hepatic fibrosis

All the livers without angiosarcomas and the uninvolved portions of the livers with angiosarcomas showed the same basic changes, the extent of which varied widely within each specimen. Excess fibrous connective tissue caused variable enlargement of most portal tracts (Fig. *3A*). A distinctive feature was the tendency for single hepatocytes and groups of hepatocytes at the margins of the portal tracts to be separated from adjacent hepatic cord cells and surrounded by this progressive fibrosis. Proliferation of bile ducts was noted in these fibrotic portal tracts together with a variable infiltration of lymphocytes. Occasionally, the walls of portal-vein branches showed focal fibrosis. Adjacent portal tracts were often connected by connective-tissue septa in the sense of a perilobular fibrosis and some thin septa traversed the parenchyma and extended irregularly toward central veins. In two cases, both with angiosarcomas elsewhere in the liver, these septa separated parts of the parenchyma to produce nodules. The hepatic capsule showed focal thickening and nodular fibrotic areas that often extended into subcapsular hepatic tissue to connect with adjacent portal tracts. The sinusoidal lining cells were focally increased in number, especially in areas where sinusoids were dilated. Intralobular, perisinusoidal fibrosis, though inconspicuous in hematoxylin-eosin-stained sections, could be demonstrated in these areas by special stains for reticulin and collagen fibers. In other areas, groups of hepatocytes, with no particular intralobular localization, varied conspicuously in cellular and nuclear size, many of them being distinctly enlarged (Fig. *3B*). Binuclear and multinucleate hepatocytes, some with much cytoplasm, were intermixed with a few hepatocytes that were smaller than normal. Degeneration and necrosis of hepatocytes, although present, were not more conspicuous than in any routine surgical specimen. In the second biopsy specimen, obtained in one patient two years after cessation of exposure to vinyl chloride, these abnormal changes in both hepatocytes and sinusoidal lining cells had disappeared, but the capsular, portal-tract and intralobular fibrosis persisted.

Figure 2. Focal Dilatation of Sinusoids, with Hyperplastic Hepatocytes (Arrow) and Excess Sinusoidal Lining Cells, Some with Hyperchromatic Nuclei (This Appearance Contrasts with That of Normal Sinusoids in the Right Lower Corner) (Hematoxylin and Eosin Stain × 160).

Splenic Changes

The available spleens were large, weighing 560 to 1050 g, and showed on cut section enlarged Malpighian follicles and a firm, beefy red pulp. Microscopically, the follicles were hyperplastic and had large germinal centers and wide perifollicular zones (Fig. 4). Occasionally, fresh hemorrhage was found around penicillary arteries and follicles. The red-pulp sinuses were widened and often lined by a continuous layer of cuboidal reticuloendothelial cells. The slightly thickened pulp cords contained many lymphoid and histiocytic cells as well as erythrocytes. Fibrosis of the red pulp was absent or inconspicuous, and hemosiderinophages were only occasionally seen.

Discussion

The relatively frequent development of hepatic angiosarcomas in workers engaged in the polymerization of vinyl chloride has now been established. In this report the emphasis is on a peculiar hepatic fibrosis in such workers and its relation to the angiosarcomas. This fibrosis, which appears to represent a second hepatic lesion attributable to vinyl chloride exposure, was found in the tumor-free

of the spleen suggests a stimulating effect by vinyl chloride or its metabolites on several types of hepatic and splenic cells.

The prominence of the enveloping growth pattern of the tumor cells, rather than a nodular growth pattern, is characteristic of hepatic angiosarcomas that develop after vinyl chloride exposure. Many cases of hepatic angiosarcoma of unknown cause fail to show this prominence.[18,19] On the basis of experience to date absence of this prominence in a given case militates against vinyl chloride as the origin. These observations may be of assistance in epidemiologic surveys. Enveloping features are prominent in angiosarcomas induced by thorium dioxide suspension (Thorotrast) in which large and irregular areas of fibrosis are observed.[20,21] Similar features are emphasized in angiosarcomas induced by inorganic arsenicals,[10,22] which, like vinyl chloride, may also produce inconspicuous hepatic fibrosis. Through the courtesy of Drs. P. J. Scheuer and M. Schmid, we were able to study histologically three cases of mild hepatic fibrosis with portal hypertension after long-term treatment of psoriasis with Fowler's solution, two of them reported.[14] We observed hepatic changes similar to those in the patients exposed to vinyl chloride — namely progressive portal fibrosis, mild intralobular fibrosis and focal hypertrophy and hyperplasia of hepatocytes.

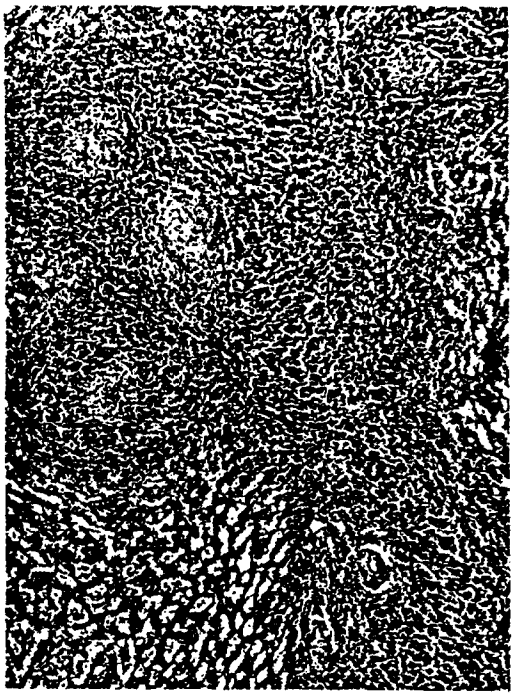

Figure 4. Surgically Removed Spleen of a Worker with Moderate Hepatic Fibrosis.

Note hyperplastic Malpighian follicles, which merge and exhibit large germinal centers. Sinuses are widened, and red-pulp cords are slightly thickened (hematoxylin and eosin stain × 40).

Inconspicuous hepatic fibrosis accompanied by splenomegaly and portal hypertension is the syndrome described by Banti.[4] An identical clinical picture is observed in some workers exposed to vinyl chloride. In contrast to splenomegalic cirrhosis, in which the splenic enlargement is caused by the hemodynamic alterations in the cirrhotic liver, in Banti's syndrome the splenomegaly precedes the hepatic fibrosis or is associated with inconspicuous fibrosis. It may, however, be followed by cirrhosis eventually.[23] Most cases of Banti's syndrome are associated with portal hypertension and are now usually designated as idiopathic portal hypertension.[24] Some authors associate the portal hypertension with a primary[25] or secondary[26] alteration of the wall of the portal vein or its branches (hepatoportal sclerosis), but this lesion was not conspicuous in our cases. Conditions resembling Banti's syndrome have been produced in animal experiments by stimulation of splenic lymphoid and reticuloendothelial cells.[27] It is possible, therefore, that one of the results of prolonged exposure to vinyl chloride may be splenomegaly due to stimulation of splenic cells, with a consequent increase in splenic and hepatic blood flow. Portal hypertension might be the result of an inability to accommodate this increased hepatic blood flow because distention of portal-vein branches is prevented by the portal fibrosis, and that of the sinusoids by the capsular and subcapsular fibrosis.

Whatever the origin of the idiopathic portal hypertension or Banti's syndrome, its apparent induction by prolonged exposure to vinyl chloride or inorganic arsenicals suggests that unidentified chemicals may cause other similar conditions in which the cause is unknown. Such cases are observed sporadically in the Western world[28,28] but are rather frequent in India,[28,29] Uganda,[30] North Africa[31] and Japan, where they are studied extensively.[32] This geographic distribution is an additional reason for suspecting environmental factors. In Banti's syndrome of unknown origin development of angiosarcoma has not been reported, but this fact does not negate the possibility that chronic exposure to vinyl chloride or inorganic arsenicals will produce either Banti's syndrome or hepatic angiosarcoma or both.

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Immunological mechanisms in the pathogenesis of vinyl chloride disease

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Summary

Vinyl chloride (VC) disease is a multisystem disorder incorporating Raynaud's phenomenon, acro-osteolysis, thrombocytopenia, portal fibrosis, and hepatic and pulmonary dysfunction. Immunological and immunochemical investigations showed the presence of circulating immune complexes in 19 out of 28 patients with the disease and in a further two out of 30 workers exposed to VC. The immunological data were reviewed in relation to the clinical picture of the disease and to the available evidence on the metabolism of VC. The results suggest that VC disease is an immune complex disorder and that the immune response is initiated by the adsorption of VC or a metabolite on to tissue or plasma protein.

Introduction

Vinyl chloride (VC) is known to be oncogenic,[1 2] but hepatic angiosarcoma may not be the most serious of its medical hazards. Acro-osteolysis was first described in 1966 in workers employed in the polymerisation of VC,[3] and since then other cases have been described.[4 5] In all these reports the affected workers helped in cleaning the autoclaves after the polymerisation process. Stewart *et al*,[6] however, reported a case in a worker who had never done this job. Their report widened the possible exposure to toxic levels of VC to other workers in the industry. The descriptions of acro-osteolysis do not describe adequately the full extent of the disorder as it may affect VC workers. The syndrome for which Lange *et al*[7] suggested the name vinyl chloride disease includes sclerotic changes in the skin, osteolysis, circulatory disturbances, thrombocytopenia, portal fibrosis, and impaired hepatic and pulmonary function.

Despite considerable effort in many countries little is understood about the aetiology and pathogenesis of VC disease. We therefore outline the immunological investigations performed on 58 workers from a VC polymerisation plant. The results explain the pathogenetic mechanisms of the disease and allow some conclusions to be drawn about its aetiology.

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Patients

The 58 patients were referred to a hospital clinic from a single VC polymerisation plant. They represented 18% of the past and present work force (320). Referrals of those still working at the plant were from either the factory medical officer or local general practitioners; ex-employees were referred after investigations made by the Employment Medical Advisory Service. For the purposes of the study the patients were divided into four clinical groups.

Group 1 (9 patients)—These patients were disabled by pain in limbs and hands and by dyspnoea. All had symptomatic Raynaud's phenomenon, which in five was clinically demonstrable; the others had severely cold hands. Four had scleroedema of the hands or face, four a mild clawing deformity of the hands, and two mild radiological evidence of acro-osteolysis.

Group 2 (19 patients)—This group was moderately disabled and multisymptomatic, excessive fatigue, limb pain, and paraesthesiae being the most common complaints. Raynaud's phenomenon was symptomatic but never observed, although many patients were found to have cold hands or feet. Scleroedema-like changes of the face were evident in two patients. No radiological abnormalities were seen.

Group 3 (25 patients)—These patients were not disabled and were continuing in active employment. They presented with miscellaneous symptoms that were not confirmed by visible abnormalities or overt clinical signs.

Group 4 (5 patients)—This group was asymptomatic, the patients having been referred because of expressed concern or because they wished to seek other employment.

The patients' ages ranged from 23 to 59 years with a mean of 39·7 years. There was no significant difference between the groups. The duration of exposure to VC varied from six to 75 months with a mean of 39 months. As the degree of exposure varied with the job at the plant the patients were divided into high- and low-exposure groups, the former being workers in the reactor building and dry-bagging

plant, and the latter maintenance fitters and warehousemen. Fifty of the patients (86%) had at sometime worked in the reactor building or dry-bagging plant. Altogether 46 patients (79%) admitted to having suffered from VC narcosis on at least one occasion; the incidence of admitted narcosis tended to decrease with diminishing severity of the disease—group 1, 100%; group 2, 80%; group 3, 76%; group 4, 60%. The differences do not approach statistical significance.

Methods

Immunoglobulins were estimated by an automated immunoprecipitin technique. *Complement* was determined in fresh EDTA plasma, C3 and C4 being estimated by single radial immunodiffusion and conversion assessed by two-dimensional immunoelectrophoresis. *Cryoprotein* studies were performed on warm-separated citrated plasma, the cold aliquot being kept at 4 C for seven days before separation of the cryoprecipitate. *Rheumatoid factor* was determined by passive haemagglutination of sensitised cells, and the presence of *antitissue antibodies* by indirect immunofluorescence.

Lymphocyte transformation was assessed by a modification of the technique of Schellekens and Eijsvoogel.[7] Lymphocytes were separated from heparinised whole blood by a Ficoll-based centrifugation method.[8] Duplicate culture tubes were set up containing culture medium, autologous plasma, and either 0·2 μg purified phytohaemagglutinin (PHA) (Burroughs Welcome) or 2 μg purified protein derivative of tuberculin (PPD). Control cultures were set up using lymphocytes from healthy donors. All cultures were incubated at 37 C for 72 hours (PHA) and 120 hours (PPD). Transformation was assessed by the incorporation of tritiated thymidine into active DNA synthesis. Similar cultures were set up with test lymphocytes supplemented with normal plasma and normal lymphocytes supplemented with test plasma to assess possible plasma inhibitory effects. *Lymphocyte subpopulations* were defined as spontaneous E-rosette-forming cells or T cells[9] and as membrane-fluorescent cells or B cells.[10] Absolute numbers of T and B cells were determined as a percentage of the total lymphocyte count. *Biopsy specimens* were snap-frozen and cryostat sections stained for IgG, IgA, and IgM, C3 and C4, and fibrinogen/fibrin by direct immunofluorescence.

Results

The major abnormalities observed were a polyclonal increase in one of the immunoglobulin classes, usually IgG; the presence of mixed cryoglobulins, including IgG, C3, and fibrinogen; and in-vivo conversion of both C4 and C3. Table I shows the mean values and ranges of immunoglobulins, C3, C4, and lymphocytes for each clinical group together with the numbers of patients with cryoproteins and showing C3 conversion. The abnormalities were present in almost all cases in group 1, and showed a progressively decreasing incidence in the other groups. The presence of circulating immune complexes was inferred when there was evidence of mixed cryoglobulins, in-vivo conversion of complement, and depressed values for C3 or C4; the presence of immune complexes showed a close relationship with clinical grouping, with 88%, 58%, 8%, and 0% respectively for the four groups. Eight of the 58 patients were classified as of low exposure, six of them being in group 2. No abnormalities were detected in these six patients. When the low-exposure workers are removed the incidence of circulating immune complexes in group 2 (85%) closely approximates that in group 1.

Autoantibody screening (table II) showed no abnormalities except for a high incidence of antinuclear antibodies in group 1. These were all of low titre (IgG 1/20-1/50) and probably resulted from tissue damage rather than an autoaggressive disease process. The only other observation of note was the presence of thyroid autoantibodies in three patients in group 2.

There was evidence of a reduction in the T cell population with a slight increase in B cells; this was most pronounced in group 1 but was also evident in group 2 and to a less extent in group 3. In-vitro stimulation showed few abnormalities except for an apparently increased frequency of reduced or absent responses to PPD. PHA transformation studies showed minor degrees of suppression, and inhibition by plasma was not seen.

Direct immunofluorescence studies of biopsy specimens of skin (10 cases), muscle (one case), and lung (one case) showed aggregates of IgG, C4, C3, and fibrinogen/fibrin in the lumen of vessels and adherent to the vascular endothelium. IgG, C4, C3, and fibrin were also detected in the media and subintimal regions of small and medium-sized arterioles.

Discussion

The major immunological features of VC disease in this series are hyperimmunoglobulinaemia, cryoglobulinaemia and cryofibrinogenaemia, and in-vivo conversion of complement involving the classic activation pathway via C4 and C3. There was additional evidence of a cellular disturbance in the form of a reduced T cell population and a modest increase in B cells. The presence of non-organ-specific antitissue antibodies in those most severely affected clinically may have related to tissue damage rather than to a pathogenetic mechanism.

Immunofluorescence studies of the biopsy material confirmed the immunochemical evidence of circulating immune complexes with the identification of immunoglobulin, complement, and fibrin/fibrinogen in the lumen of vessels, adherent to endothelium, and in the vessel wall. An immune complex or cryoglobulinaemic vasculitis would be consistent with the findings of a perivascular inflammatory infiltrate as described by Markowitz

TABLE I—*Mean values and ranges for immunoglobulins, complement, and lymphocytes in each group and numbers of patients in each group with cryoproteins and showing C3 conversions*

Group	No of patients	Immunoglobulins (g/l)			No with cryoglobulins	No with cryofibrinogen	Complement (g/l)		No with complement C3 conversion	Lymphocytes × 10⁹/l		
		IgG	IgA	IgM			C3	C4		Total	T cells	B cells
1	9	14·3 (10·0-19·0)	2·8 (1·8-4·7)	1·3 (0·4-2·7)	8	5	1·0 (0·6-1·5)	0·45 (0·19-1·10)	6	1·8 (1·0-3·1)	0·4 (0·1-1·5)	0·6 (0·1-1·5)
2	19	11·9 (8·3-18·5)	2·4 (0·6-5·0)	0·9 (0·3-2·1)	13	13	0·8 (0·6-1·3)	0·38 (0·22-0·7)	9	2·4 (1·3-5·4)	0·5 (0·1-1·8)	0·7 (0·3-1·5)
3	25	9·5 (6·8-11·4)	2·0 (0·9-4·1)	0·9 (0·4-1·9)	2	1	0·8 (0·5-1·2)	0·45 (0·32-0·69)	10	2·0 (0·7-3·0)	0·4 (0·1-1·0)	0·5 (0·1-0·8)
4	5	12·1 (9·5-16·0)	3·6 (1·1-5·2)	1·0 (0·5-1·4)			0·8 (0·6-1·0)	0·38 (0·27-0·46)	3	2·5 (2·0-3·0)	0·4 (0·3-0·4)	0·5 (0·3-0·6)
Normal values		9·5 (6·0-14·0)	1·8 (0·5-3·0)	0·9 (0·5-1·7)			1·2 (0·8-1·7)	0·45 (0·30-0·75)		2·0 (1·5-2·5)	1·0 (0·6-1·5)	0·7 (0·4-1·0)

TABLE II—*Results of autoantibody screening in the four groups of patients*

Group	No of patients	No with rheumatoid factor	No with antitissue antibodies to:				
			Nuclei (ANA)	Mitochondria (AMA)	Smooth muscle	Gastric parietal cells	Thyroid
1	9		8		2		
2	19				1	1	3
3	25	1	2		1		
4	5	1					
Total	58	2	10		4	1	3

et al,[4] although the severe narrowing of dermal vessels with subintimal fibrosis described by Harris and Adams[4] could be construed as an end-stage phenomenon of a similar process. Both histological features were present in this series, although the subintimal proliferation was seen only in the cases of longest duration. The tendency towards thrombocytopenia noted in many patients could be construed as confirmatory of an immune complex disorder with complement activation.[12] The persistence of disease activity as evidenced by the presence of cryoprecipitate and conversion of complement in workers who had been removed from exposure to VC for more than six months is in accord with the observations of Veltman *et al*.[13]

Despite these pronounced abnormalities a search of published work on VC disease failed to disclose any similar investigation, most reports having concentrated on the hepatotoxic effects of VC and its oncogenic properties. Danishevsky and Egorev[14] showed that tissue damage from VC could produce allergenic substances. Hervieux and Tessier[15] had shown that a chemical dermatitis could be induced among workers in the industry. One patient in group 1 complained of skin irritation a year before, with evidence of Raynaud's phenomenon and dyspnoea, and gave a positive reaction to a patch test with polyvinyl chloride (PVC) powder. One other patient (group 3) had also complained of severe generalised pruritus.

In a detailed study of 13 patients employed in PVC production with circulatory disturbances, thrombocytopenia, splenomegaly, and hepatic malfunction, Lange *et al*[7] concluded that there was no evidence for an autoaggressive disorder. A more recent report[12] detailed the investigation of 70 patients from a work force of 128 at a single plant, the findings of which did not change the earlier conclusions. These workers did make the point, however, that although skin and bone changes may disappear when the patient is removed from contact with VC, the thrombocytopenia may persist for 12-18 months after exposure.

In a study of 36 workers exposed to VC who were shown to have leucopenia, thrombocytopenia, and splenomegaly, Suciu *et al*[16] showed an increase in the γ-globulin in 11. Although they found only a 6% and 2·9% incidence of clinical Raynaud's phenomenon in two series of VC workers, they noted that the phenomenon cleared spontaneously on removal from exposure and that the different incidence figures were associated with a 22-fold decrease in VC levels. They also quoted a much higher (66% and 55% respectively) incidence of vasospastic changes in discussing the significance of the splenogmealy, they suggested that VC acted as an irritant in the reticuloendothelial system to produce a reactive splenic enlargement. Marsteller *et al*,[17] in an extensive investigation of 50 VC workers, introduced some immunological tests into the initial protocol but abandoned them when they failed to give positive results. No comment was made on the presence or absence of hypergammaglobulinaemia. These workers also suggested the possibility of reticuloendothelial stimulation as a cause of the splenomegaly and the hepatic littoral cell hyperplasia, although they regarded the latter as a possible pathogenetic factor in the development of hepatic angiosarcoma.

The metabolism of VC in man is not fully understood. Hefner *et al*[18] showed that in rats metabolism is by the alcohol dehydrogenase pathway at low concentration, and via an intermediate chloroethylene oxide at higher concentrations. Williamson[19] proposed that the intermediate product is more likely to be a cyclic dioxide. Both the oxide and dioxide would be highly reactive molecules and would be able to bind to free sulphydryl and amino radicals. The incorporation of these into protein synthesis would give a conformationally altered molecule that would be antigenic. The inclusion of this intermediate product with its chloride residue in a protein molecule would have a haptenic effect in the incitement of antibody synthesis. The theoretical interaction between VC and plasma proteins was partially confirmed by Oster[20] in his observations of the binding of VC or its metabolite to serum albumin.

The association of the observed results with the experimental metabolic data allows the construction of a theoretical model for the pathogenesis of VC disease and explains many of the features of this multisystem disorder. A metabolic product of VC, presumably the dioxide, binds to plasma protein, producing either a haptenic group or a conformational change within the protein molecule. This acts as an antigen that escapes tolerance and stimulates B cell proliferation and immunoglobulin production. The antibody and antigen interact to produce a soluble complex that is both cryoprecipitable and capable of initiating the complement sequence by the classic activation pathway. The cryoprecipitate and reactions secondary to complement activation will produce platelet aggregation and apparent thrombocytopenia, fibrinogen/fibrin conversion, and vascular occlusion. The vascular occlusion, either temporary or permanent, is enough to explain the observed clinical, radiological, and histological findings in the skin, skeletal and soft tissues, and lungs. By producing ischaemia the vascular occlusion would also stimulate new collagen biosynthesis, as observed by Jayson *et al*.[21] This final episode in the pathogenesis of the disease process would then be further augmented by the interaction of collagen and complement,[22 23] resulting in further activation of the complement pathway and thus reinforcing the terminal recycling phenomenon.

The frequency with which abnormalities were detected in exposed workers in this series, especially in those groups (2 and 3) in which there were few or no overt clinical signs, suggests that the disease process may be more common than is generally supposed. Further studies are in progress to investigate the total exposed population at the factory and to study populations from other related industrial plants.

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BASIC ARTICLES
LIVER DAMAGE
CARCINOGENESIS

ngiosarcoma of the Liver in
inyl Chloride/Polyvinyl Chloride Workers

977 Update of the NIOSH Register

ert Spirtas, Dr. P.H., and Rose Kaminski, M.S. Hyg.

the December 1974 issue of the Journal of Occupational dicine (16:809), NIOSH presented data on 26 cases of liver giosarcoma among vinyl chloride workers, 21 of whom rked in the polymerization process. The present update pro- es details on 64 cases of angiosarcoma among vinyl chloride merization workers reported as of October, 1977. Con- ed surveillance of these workers is encouraged for the pur- e of furthering our knowledge of this disease process in tion to vinyl chloride exposure.

Since the original publication of NIOSH's register of 26 cases of angiosarcoma associated with occupational exposure to vinyl oride or polyvinyl chloride (VC/PVC),[1] evidence has continued ccumulate which further confirms this association. In the May issue of *JOM*,[2] an additional 12 cases were presented. Since update, 33 more cases have been reported to NIOSH and one has been discounted (U.S. Case No. 14) as of October, 1977. s yields a total of 70 cases known to NIOSH, 64 of whom were merization workers. Details of the 64 cases among poly- rization workers are provided in Table 1. Data on non-poly- rization workers is not presented in this update since there has n essentially no change. (One West German case was added the previously published table of six cases among non-poly- rization workers,[2] while one U.S. case (No. 14) was removed ause the diagnosis could not be confirmed.)

Table 1 consists of 23 U.S. cases and 41 foreign cases from 11 rent countries. The 23 cases identified among U.S. poly- rization workers represents approximately 10% of all liver giosarcomas identified in the U.S. since 1964. This percentage mate is based on the number of cases identified by the Chronic ases Division, Bureau of Epidemiology, CDC, through their ionwide case finding effort for liver angiosarcoma for the years 1964-1974.* In the course of their study, CDC identified approximately 240 cases (confirmed by a panel of pathologists), of which 75% occurred during the 1964-1974 study period.

Some of the information presented in Table 1 is summarized below. The numbers in parentheses represent the medians which were calculated from the data in the previous update.[2] (1) The ages at diagnosis ranged from 37 to 71 years with a median of 49 years (44 years). (2) The latency periods, i.e., years from first exposure to diagnosis, ranged from nine to 38 years with a median of 21 years (17 years). (3) The total years exposed ranged from four to 31 years with a median of 18 years (16 years).

A distribution of the cases by date of diagnosis is presented in Fig 1. Although it is difficult to interpret the aggregation of differing sources of data, and while the data for 1977 is incomplete, this figure suggests a gradual increase over time in the reported cases of VC-induced angiosarcoma. During the past 17 years (1961-1977), a total of 23 cases have been identified in U.S. polymerization workers. Fifty-two percent of these cases had been diagnosed over the past five years. The rise in the number of cases diagnosed in these workers during the 1970's is probably the reflection of two events: (1) the increased surveillance of VC/PVC workers during this time and (2) the growth of the VC/PVC industry after World War II. The 20 to 30 year lag between the growth of the industry after World War II and the increase in the number of diagnosed angiosarcomas among VC/PVC workers observed during the 1970's roughly coincides with the median latency period (21 years).

In regard to some of the observations made from the data in Table 1, it will be interesting to note whether certain trends continue. For instance, the age at diagnosis and latency period appear to be increasing. Some possible explanations for these phenomena are (1) the initial cases may have had heavier exposures, (2) the ini-

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*The results of CDC's case finding effort were obtained through personal communication with Dr. Henry Falk, Chronic Disease Division, Bureau of Epidemiology, CDC, Atlanta, Georgia.

Table 1. — Reported Cases of Angiosarcoma of the Liver Among Vinyl Chloride Polymerization Workers.

Country	Case No.	Birth Date	1st VC or PVC Exposure	Diagnosis of Angiosarcoma	Age at Diagnosis	Years from 1st Exposure to Diagnosis	Total Years of Exposure	Date of Death
Belgium	01	00-00-00	00-00-00	00-00-00	00	00	00	06-29-76
Canada	01*	12-15-13	00-00-44	00-00-55	41	11	11	09-02-55
Canada	02*	03-06-14	00-00-43	00-00-57	43	14	14	12-21-57
Canada	03*	08-26-19	00-00-41	00-00-62	42	21	20	03-22-62
Canada	04*	04-05-19	00-00-45	00-00-67	48	22	22	01-21-68
Canada	05*	05-07-11	00-00-44	00-00-68	57	24	05	07-05-68
Canada	06*	12-15-19	00-00-47	00-00-71	51	24	23	04-10-71
Canada	07*	11-09-19	00-00-48	00-00-72	53	25	25	12-24-72
Canada	08	05-13-20	00-00-61	00-00-73	53	12	05	06-12-73
Canada	09	07-19-21	00-00-46	00-00-74	53	28	26	09-04-74
Canada	10	05-16-15	00-00-53	00-00-76	61	23	14	04-00-77
Czechoslovakia	01*	00-00-28	00-00-57	00-00-73	46	16	16	00-00-74
Czechoslovakia	02*	00-00-26	00-00-51	00-00-66	40	15	15	00-00-66
Fed. Rep. Germany	01*	06-04-30	10-01-56	09-19-68	38	12	12	01-25-69
Fed. Rep. Germany	02*	07-26-31	10-14-57	09-25-70	39	13	12	12-14-71
Fed. Rep. Germany	04	09-04-30	04-16-57	00-00-74	44	17	17	11-25-74
Fed. Rep. Germany	05*	01-01-32	12-16-62	00-00-75	43	13	12	01-09-75
Fed. Rep. Germany	07*	09-29-26	04-15-54	00-00-75	49	21	12	11-13-75
Fed. Rep. Germany	08*	10-19-17	04-19-54	00-00-75	58	22	21	12-25-75
Fed. Rep. Germany	09*	12-13-34	12-02-59	06-16-76	42	17	15	Alive
Fed. Rep. Germany	10*	07-25-29	10-10-55	06-28-77	47	22	22	06-28-77
Fed. Rep. Germany	11*	12-29-36	01-02-61	00-00-77	41	16	10	03-07-77
France	01*	04-15-24	01-00-46	02-18-67	43	21	19	02-19-67
France	02	06-03-11	07-06-59	01-08-75	63	15	12	01-24-75
France	03*	00-00-19	00-00-46	01-00-75	55	29	29	06-29-75
France	04*	01-27-27	10-19-49	01-04-76	49	26	26	01-04-76
France	05*	01-29-38	00-00-65	04-00-76	38	11	10	05-13-76
France	06*	04-14-34	00-00-58	09-00-76	42	18	17	09-12-76
France	07	00-00-27	07-01-50	07-00-76	49	26	23	07-02-76
France	08*	04-01-34	05-23-57	12-03-76	42	19	19	01-30-77
Great Britain	01*	04-20-01	00-00-44	12-00-72	71	28	22	12-00-72
Great Britain	03	06-02-37	02-00-66	12-00-74	37	09	04	12-24-74
Italy	02*	11-13-29	00-00-67	12-13-72	43	15	06	12-00-72
Italy	03*	03-14-20	00-00-53	07-10-75	55	22	21	07-10-75
Japan	01	08-01-22	04-00-53	08-21-74	52	22	22	10-24-75
Norway	01*	12-23-15	03-00-50	12-20-71	56	22	21	01-04-72
Sweden	01*	06-23-27	08-14-51	08-00-74	43	19	18	10-20-70
Sweden	03*	06-10-10	05-00-47	03-19-76	65	29	21	03-19-76
Sweden	04*	11-16-14	00-00-46	05-12-77	62	31	31	05-12-77
U.S.A.	01*	10-17-23	12-09-48	03-03-73	49	24	21	03-03-73
U.S.A.	02*	08-19-33	11-15-55	05-00-70	37	14	13	09-28-71
U.S.A.	03*	05-25-15	11-28-45	12-19-73	58	28	28	12-19-73
U.S.A.	04*	01-15-24	07-06-52	08-19-67	43	15	15	01-07-68
U.S.A.	05*	01-25-12	06-19-44	04-09-64	52	20	20	04-09-64
U.S.A.	06*	11-23-28	01-17-62	02-00-74	46	12	12	07-24-75
U.S.A.	07*	05-03-22	08-27-44	00-00-68	45	24	17	03-23-68
U.S.A.	08*	05-06-20	10-07-46	08-00-61	41	15	15	08-29-61
U.S.A.	09*	11-08-31	05-28-45	03-01-74	43	29	24	03-00-75
U.S.A.	10*	08-16-13	06-12-51	05-00-68	55	17	17	05-10-68
U.S.A.	11*	05-27-09	10-14-46	03-00-70	61	23	23	03-18-70
U.S.A.	12*	11-17-18	09-13-49	05-02-69	50	20	19	05-02-69
U.S.A.	13*	12-01-21	12-11-42	05-00-74	52	32	26	07-04-74
U.S.A.	16*	11-04-27	05-08-50	00-00-69	41	19	04	03-27-69
U.S.A.	17*	05-05-31	06-23-55	10-11-74	43	19	19	Alive
U.S.A.	18*	04-22-28	09-15-54	00-00-75	46	21	11	11-02-75
U.S.A.	19*	00-00-15	00-00-43	06-19-75	60	32	22	04-06-76
U.S.A.	20*	08-31-17	00-00-55	01-30-76	58	21	18	01-30-77
U.S.A.	21*	09-02-09	12-00-46	00-00-77	67	30	21	01-02-77
U.S.A.	22*	10-02-23	07-11-47	01-00-76	52	29	28	12-04-76
U.S.A.	23*	00-00-23	09-0-58	04-06-73	50	15	14	04-06-73
U.S.A.	24*	05-07-17	00-00-39	05-27-77	60	38	26	05-27-77
U.S.A.	25*	08-07-10	02-00-47	03-10-77	67	30	20	03-10-77
Yugoslavia	01*	04-05-14	00-00-53	04-08-73	59	20	20	04-08-73
Yugoslavia	02*	11-15-31	00-00-50	07-12-73	42	23	18	07-12-73
Total Reported Cases 64								

*Diagnosis was microscopically confirmed
"00" indicates unknown data

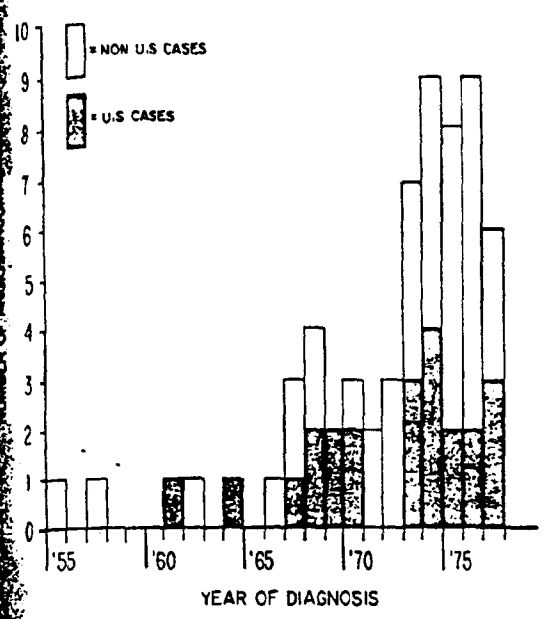

Fig 1. — Number of cases of VC/PVC related angiosarcomas reported to NIOSH by year of diagnosis. (This represents only 63 of the 64 cases known to NIOSH since information on diagnosis is missing for one case.)

...al cases were more biologically susceptible, or (3) random fluctuation. Analysis of the latency period for this disease in vinyl chloride workers should be a fruitful area for future study since multiple etiologies do not seem to be a factor in these cases and the exposure periods can be fairly well defined via employment histories.*

*Recent preliminary findings by NIOSH's Division of Biomedical and Behavioral Science suggest that the interaction of disulfuram with the enzymatic detoxification of ethylene dibromide results in increased carcinogenicity (including angiosarcoma of the liver) in laboratory rats.

One of the values in maintaining this register of cases among vinyl chloride workers is that it provides a base from which hypotheses for future research can be generated. However, the extremely rare occurrence of liver angiosarcoma lends great importance to the need for as complete reporting of the cases as possible if we are to develop a better understanding of liver angiosarcoma in relation to vinyl chloride exposure. The occupational health community is asked to report all new or as of yet unreported cases of angiosarcoma among VC/PVC workers to either Dr. Robert Spirtas or Ms. Rose Kaminski, Surveillance Branch/DSHEFS/NIOSH, 4676 Columbia Parkway, Cincinnati, OH 45226, (513) 684-3284.

In addition to vinyl chloride's association with angiosarcoma of the liver, recent studies have also found vinyl chloride to be associated with excesses in brain cancer and neoplasms of the respiratory and lymphatic systems.[3,4,5] More studies are needed to assess these findings. For an overview of the literature concerning the health effects associated with VC/PVC exposure, the reader is referred to Volume 246 of the Annals of the New York Academy of Sciences.[6]

The authors wish to thank Dr. J. William Lloyd, Dr. Henry Falk and Mr. Richard Waxweiler for their constructive criticisms.

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Angina as a Diagnostic Predictor

It turns out that a history of typical angina is as good a predictor of ischemic heart pain or coronary disease as any other data we can get, including the exercise electrocardiogram. If you get this typical history your chances of finding significant obstructive coronary disease are 80 or 90%, depending, of course, on the "expertise" of your evaluation. If you find atypical features, on the other hand — something isn't quite right and you're not sure it's really angina — then the chances are going to fall off to only about 50% that such patients will have obstructive coronary disease. And if you're pretty certain that this is not ischemic pain, then the incidence of coronary disease falls to about 10 or 20%. So the ability to take a history and identify the typical anginal syndrome is your best way of determining whether the patient is likely to have obstructive coronary disease. We're all anxious to use exercise electrocardiography and other studies as supplements, but there's no specific test available that is any better than a good and careful history.

— From "Bedside Cardiac Diagnosis" by J. O'Neal Humphries, in *Emergency Medicine*, January, 1978.

Vinylchloride induced hepatic angiosarcoma

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The relationship between vinylchloride exposure and human angiosarcoma of the liver (ASL) received attention in 1973 when a case of this rare tumour was diagnosed at autopsy[1].

Case report

A 39-year old man was first seen in July 1979 with pain in the right upper quadrant. The liver was palpated at the right costal margin. Oral cholecystography and barium swallow were normal and liver function tests were in normal limits. From 1965 to 1970 he had cleaned reactors used for the polymerization of the vinylchloride monomer in PVC and was thus exposed to high amounts.

He was admitted 3 months later with persisting right upper quadrant pain, loss of appetite and fatigue. The liver edge was by then hard and 4 cm below the costal margin. The ESR was accelerated (80 mm/h) and alkaline phosphatases and GGTP were elevated. Carcino-embryonic antigen (CEA) and α fetoglobulin were normal. Ultrasound showed a large dense tumour of the right hepatic lobe with areas of necrosis. The ^{99m}Tc hepatic scan confirmed the presence of an ill-defined filling defect in the inferior part of the right lobe with two small defects at the level of the hilum.

The liver computerized tomography confirmed the integrity of the left lobe.

A selective angiography of the celiac artery revealed a hypervascularized tumour of the right hepatic lobe (*Figure 1*).

At a right thoracophrenolaparotomy the tumour was found to be confined to the right lobe. An extended right lobectomy was then performed. The postoperative recovery was uneventful and the patient was sent home with a monthly administration of Vincristine (1 mg IV) and Adriamycin (150 mg/m² IV) which was discontinued in June 1981.

Repeated controls up to September 1981 by liver scan and computerized tomography remained normal. The patient is still in good health 38 months after the resection.

Pathology

The resected specimen was 2050 g. On macroscopical examination, the main tumour (13·5 × 8 cm) was yellowish and spongy and contained cystic and haemorrhagic zones. A second smaller haemorrhagic mass was found at the inferior aspect of the right lobe surrounded by numerous purple masses.

Macroscopically, the main tumour showed large areas of necrosis and haemorrhagic pseudocystic spaces (*Figure 2*). These spaces were surrounded by areas of dense vascular proliferation. The sinusoids were lined with variably sized irregular sarcomatous cells with hyperchromatic nuclei. Elsewhere, blood-filled spaces were surrounded by sarcomatous cells. The sarcoma cells encompassed adjacent liver cells and bile ductules and infiltrated the parenchyma. The pathological diagnosis of multicentric angiosarcoma was made. The rest of the liver was normal except for some moderately enlarged portal tracts. Progressive fibrosis separated hepatocytes at the margins of the portal tracts from adjacent hepatic

Figure 1 *Selective angiography of the coeliac artery. The hypervascularized tumour is supplied by an anterior branch (arrow) of the right hepatic artery*

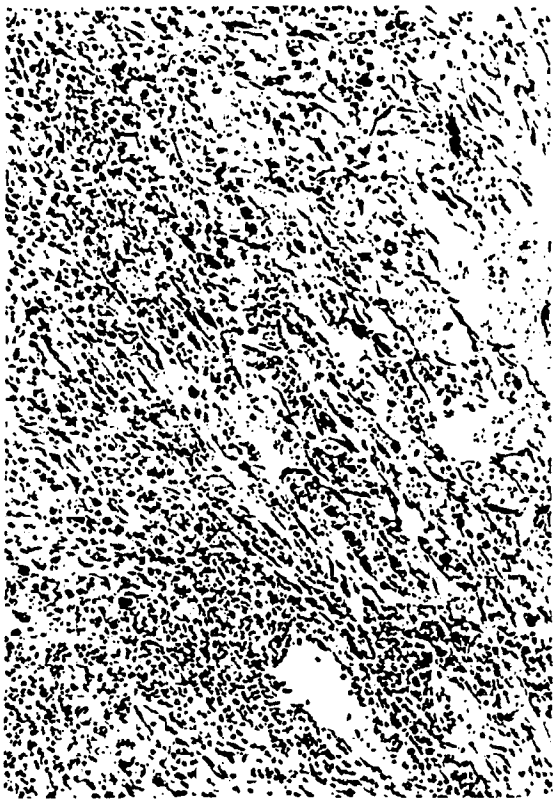

Figure 2 *Photomicrography of the main tumour showing blood filled spaces surrounded by sarcomatous cells. (Haematoxylin eosin, ×160)*

cord cells. Anisocaryosis and anisocytosis were frequent. A cross-section biopsy of the left lobe showed normal tissue with slight hepatocytic anisocaryosis. The lymph nodes taken from the liver hilum were hyperplastic. The main features of this tumour were its multicentricity and the presence of mild fibrosis.

Discussion

The occurrence of liver angiosarcoma in vinylchloride polymerization workers was reported in 1974[1,2]. Prolonged exposure and long interval from initial exposure is required before liver disease becomes apparent. The average interval is 12 years (range 6–29)[3]. Our patient was exposed for 5 years and became symptomatic 14 years later.

It is a rapidly progressing fatal disease, especially in adults. The clinical features include rapid liver enlargement with haemorrhagic ascites, fast deterioration and cachexia usually with death within 6 months.

The treatment is disappointing and chemotherapy and radiation of palliative value only. If the diagnosis is made early, the disease is localized and there is no associated liver fibrosis or portal hypertension, resective operation might prove to be curative. However, there are few reported cases of successful operative removal of hepatic angiosarcoma and the longest survival has been 16 months[4].

In our case the tumour was confined to the right lobe and there was no sign of the extensive fibrosis. So far the patient is apparently free of disease after 38 months. This is, to our knowledge, the longest published survival.

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Brachiodistal vein arteriovenous fistula with valve destruction as a secondary access procedure for haemodialysis

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With the increasing use of dialysis and the length of time which has passed since programmes began, the number of patients experiencing problems with access are either increasing or will increase in the future. It was hoped that the wide variety of artificial grafts would overcome the access problem by allowing their prolonged use when primary fistulae failed. Unfortunately, the longterm patency of these grafts is in some doubt[1,2], and consequently wherever possible the patient's own vessels should be used[3,4]. This paper describes a technique which can be useful in achieving this aim where other methods have failed.

Method

The patient is usually one who has had several previous attempts at fistula creation in the wrist or elsewhere. No obvious vessels are available, and the first step is to assess the veins carefully by a combination of clinical examination after placement of appropriate light tourniquets on the arm, and superficial venography, which will often show impalpable veins. In the unlikely event that there are no vessels available for the operation to be described, an artifical graft may have to be used.

Using an axillary block or general anaesthesia, the patient's arm is prepared from the axilla to the wrist. The brachial artery is explored through an S-shaped incision passing across the brachial skin crease. Before the artery is exposed, a search is made for any veins that may be present in the area under the incision. The best vein is the basilic, but if not available it is usually possible to find a vein though it may be some distance from the brachial artery. Once a vein has been found, the brachial artery should be exposed and the vein mobilized in such a way that approximation to the artery is possible without tension. Once this has been done, the patient is given Heparin (5000 units) and a size 3 Fogarty embolectomy catheter is passed down the vein from elbow level distally in order to define the path of the vein and its distal extremity. When the catheter has passed as far as it can go, preferably to wrist level, the vein containing it is exposed and controlled with slings. The vein at the elbow should next be turned across and anastomosed end-to-side to the brachial artery using 5/0 or 6/0 prolene. The posterior wall of the anastomosis is completed leaving the anterior wall open (*Figure 1*). Any large branches passing from the main vein deeply into the forearm near to the fistula are ligated and divided. The smallest of the valve strippers (Lépine à Lyon Ltd) is now passed through a small transverse incision made in the chosen vein near to the wrist. Upward progress of the instrument can be assessed by palpation until it reaches the anastomosis (*Figure 1*). The stripper is then gently withdrawn using a circular movement to cut through the valve leaflets. This process is repeated until the biggest stripper consistent with the size of the vein is passed without

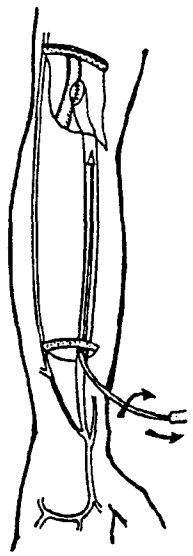

Figure 1 *The stripper is being withdrawn using a gentle circular movement to destroy the valves.*

Biology of Cancer and the Cancer Cell: Normal and Abnormal Regulation of Cell Reproduction

David M. Prescott, PH.D.

The distinguishing property of the cancer cell is its capacity to multiply and invade in situations where normal cells are restricted. The extraordinary research effort of the last decades to translate this primary difference between cancer and normal cells into unique and specific biochemical differences is based on the very reasonable premise that the discovery of a unique biochemical property for all cancer cells is virtually an essential step in the eventual design of completely and absolutely specific anticancer chemotherapeutic agents. Unfortunately, the efforts to achieve such translation have so far largely failed, but in the course of this "failure," an enormous amount has been learned about cell chemistry, physiology and reproduction.

We have learned to make a sharp distinction between abnormal cell reproduction and abnormal regulation of cell reproduction. The failure to recognize this difference has sometimes been a source of confusion, has inspired some fruitless research and has led to pointless controversy. Neoplasia is defined as the formation of any new and abnormal growth, but there is no evidence that in a neoplasm the cells grow and divide by any other than the same rules and mechanisms by which cells grow and divide in any normal tissue or in any other situation (e.g., in culture).

Thus, the abnormality in neoplasia is present not in the processes that con-

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A Spirit of Adventure:
A Personal Tribute to Wendell G. Scott, M.D. (1905-1972)

The onrushing flood of future possibilities brings with them a spirit of adventure; a need for bold, imaginative thinking; a willingness to gamble on provocative new concepts; the courage to break away from entrenched viewpoints, and the aggressiveness to bring them into reality.

—Wendell G. Scott, M.D. *The Changing Concepts in Control and Prevention of Cancer. Presidential Address to the American Cancer Society, October 28, 1964.*

It was exhilarating just being in his presence. His quick smile and spontaneous chuckle induced an atmosphere of friendship and warmth. His bright and alert look, the way he leaned forward to greet you, said that here was a man of action. He was impatient, although always courteous and diplomatic, about unnecessary delays, postponements and obstacles to rapid achievement, and a meticulous planner of the details and organization needed to get the desired results. This was Dr. Wendell G. Scott —a man of extreme capability and considerable achievement.

We greatly respected his stature in the world of medicine, yet it somehow seemed right to call him "Scottie." He was President of the American Cancer Society; Professor of Clinical Radiology, Washington University School of Medicine; President of the American Roentgen Ray Society; Editor of the journal *Cancer;* Chancellor of the American College of Radiology; Consultant to the Surgeon General with the rank of Rear Admiral in the Navy; Member of the National Cancer Advisory Board; recipient of the American Cancer Society's highest citation, the Annual National Award—and held a host of other titles and committee responsibilities which, if listed, would challenge those of lesser energy. It was said that he had 24 jobs a day—one for each hour.

When Scottie accepted an assignment for the American Cancer Society, things began to happen, and quickly. He was a man soon in motion. Telephones buzzed, visits were arranged, meetings called, committees appointed, conferences organized, documents drafted and the goals achieved in seemingly impossible short periods of time. The enthusiasm he generated for professional education, the earlier diagnosis of cancer and the use of modern technological resources captured all who were near him. Even as an intern at St. Louis' Barnes Hospital in the 1930's it was said, "Give Scottie the job—he works harder than anybody else." Most of us change with the years; Dr. Scott's appetite for hard work did not.

Although Scottie served a variety of organizations and pursued a wide range of interests, we never had the feeling that he was being shared with others. This trait of total commitment was typical of everything he chose to do, and we could always rely on him for a solid and outstanding performance. He lived by the adage, "We work not only to produce, but to give value to time."

Dr. Wendell G. Scott lost only one battle in his life—to the disease he was so eager to control.

Arthur I. Holleb M.D.

stitute growth and division, but in some degree of loss of regulation of cell reproduction. The degree of loss of such regulation is, from one type of neoplasm to another, highly variable but apparently rarely complete. This is so because neoplastic cells, with few possible exceptions, do not reach the high rates of proliferation that are quite usual for certain normal cell types (e.g., stem line cells in blood-forming tissue, or progenitor cells in certain epithelia).

Various hypotheses have been evolved based on the presumption that growth and division in a neoplastic cell are in themselves intrinsically abnormal, or that neoplasia results from altered patterns of such housekeeping activities as energy metabolism or deoxynucleoside triphosphate synthesis, but such hypotheses are clearly not in keeping with the specific experimental findings. In addition, they are not consonant with the present large body of knowledge about the biochemistry, genetics, physiology and cytology of cell reproduction. Neoplasia is the result of a heritable lesion or defect in the still unidentified mechanism that regulates the initiation of chromosome replication (i.e., the entry of a cell into the synthesis of deoxyribonucleic acid—DNA).

In cancers, the variable degree of loss of control over cell reproduction, observed as different growth rates, is accompanied by two additional, primary changes: (1) a failure to a greater or lesser degree of the cells involved to develop or maintain a fully differentiated, mature state; and (2) a loss of repression of cell migration that is expressed to a greater or lesser degree as an ability to invade normal tissues. A variety of observations point up the specific inter-relatedness of these three phenomena (i.e., loss of regulation of cell proliferation, a deficiency in differentiation, and a loss of repression of cell migration), but the elucidation of the molecular mechanisms that underlie the causality of this inter-relatedness is still to be reached. All three of these basic changes in cell behavior may be initiated by a single common causative event. It is well known that the more highly differentiated the cancer cell, the more slowly it grows, and vice versa. This is one of the observations that supports the thesis that an integral part of differentiation is the establishment of a rigid control system over cell reproduction. Incomplete differentiation of a cell—for example, of an hepatocyte—means not only absence of some normal hepatocyte properties, but includes an incomplete differentiation of regulation of cell reproduction. The greater the deficiency in differentiation, the greater the deficiency in the regulation of cell reproduction.

In basic research in cell biology, the study of the loss of regulation of cell reproduction logically proceeds from a prior understanding of the molecular basis for the intact regulatory mechanism in normal tissues. This latter understanding depends, in turn, upon a firm and detailed knowledge of the processes that constitute cell growth and cell division. The discussions in this paper deal, therefore, with the components of cell growth and reproduction that make up the life cycle of every proliferative cell, normal or abnormal. On this basis we proceed to the normal interruption of the cell life cycle by which the regulation of cell reproduction is accomplished. And finally, we consider briefly the possible basis for the loss of such regulation.

The Cell Life Cycle

All of the events of cell reproduction are encompassed within the period called the cell life cycle. The life cycle extends from the completion of one cell division to the completion of the next, and it contains all of the events necessary to produce a doubling in the structural elements and functional capacities of the cell, and—what is espe-

cially important in this discussion—it includes those events that are particularly and directly preparations for cell division. For present purposes, relatively little attention need be given to growth or development of the cytoplasm, because the pace of cell progress through the life cycle is governed principally by the nuclear events of chromosome replication and segregation (mitosis). All other growth activities are ultimately geared directly or remotely to the accomplishment of these chromosomal processes, and the regulation of cell reproduction is precisely a question of the regulation of chromosome replication.

The cell life cycle, which is divided into four subsections on the basis of chromosome replication and segregation, is summarized in the diagram. (Fig. 1.) The first subsection, which occupies the first part of interphase, is called the G_1 phase, and it extends from the completion of the previous cell division to the beginning of chromosome replication. The essence of chromosome replication is deoxyribonucleic acid synthesis, and the beginning of DNA synthesis defines the end of the G_1 phase and the initiation of the S (synthesis) phase. The S phase is usually six to eight hours in mammalian cells, and is followed by an interval of usually two to three hours, called the G_2 phase, which separates the end of chromosome replication (DNA synthesis) from the detectable beginning of prophase in mitosis and cell division. The period of mitosis or division is called the D (division) phase and commonly lasts less than an hour. The terminology of G_1, S, G_2 and D was originated by Howard and Pelc[1], who first clearly defined the existence of the four subsections of the cycle from studies on plant root cells, although it had been shown previously by others that DNA synthesis occurs during interphase.[2] Although much has been learned about the properties and contents of all four of the cycle subsections, the definitions of the G_1 and G_2 phases continue to be essentially negative (i.e., no specific molecular events have yet been discovered which define or explain the existence of these two phases). They stand as time gaps between the clearly defined D and S phases.

The transition of a cell from G_1 to S is a critical point in the cycle. Once DNA synthesis begins, a cell ordinarily proceeds quickly to the completion of division and the two resultant daughter cells enter the next G_1 phase. Cells do not normally cease reproductive activity by becoming arrested in either S, G_2 or D, although, in certain circumstances, both S or G_2, and even D, can be very long. Because control of cell reproduction is essentially always achieved by action in the G_1 phase, and never in either the S or G_2 phases, little more will be said about these latter two. There are many important unanswered questions about events within S and G_2, but these are not immediately germaine to the problem of regulation of cell reproduction. In mammalian cells, G_2 is probably concerned primarily with synthesis of the macromolecules needed for assembly of the mitotic apparatus and with the beginning of condensation of the chromosomes into their mitotic configuration.

The individual cell cycle is made up of a succession of many steps. The problem is to determine the nature of these steps and the means by which these steps are held together by cause and effect relationships in a way to produce the ordered continuity known as the cell cycle. The identification of the four major subsections of the cycle (i.e., G_1, S, G_2, and D) represents the beginning of the decipherment of this cause and effect sequence. It is likely that only a small fraction of the total genetic apparatus of a cell is concerned with these matters of continuity of the cell life cycle.

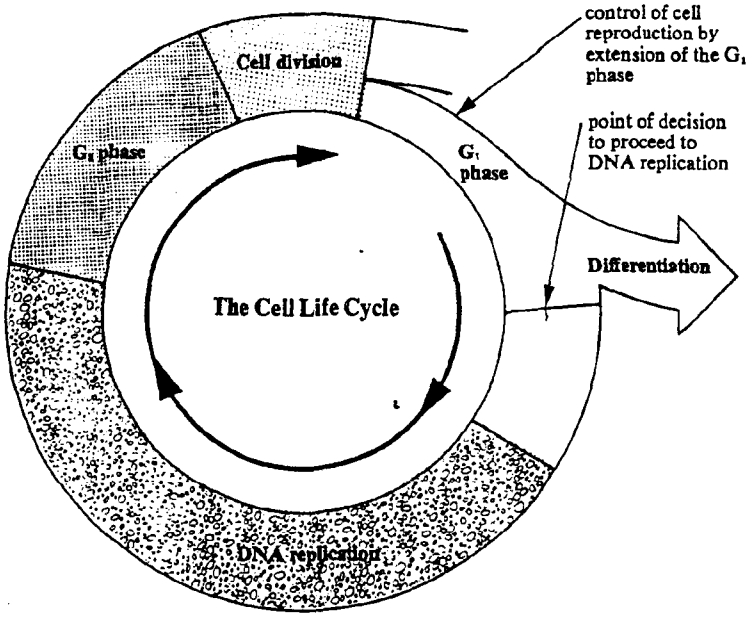

Fig. 1. A diagram of the major components of the cell life cycle. In a mammalian cell in culture, G_1 is typically 4 hours; S, about 7 hours; G_2, 2 to 3 hours; and division (D), about 1 hour. Cell reproduction in a tissue is regulated by the arrest of cells in the G_1 phase. The lesion in cancer is the failure of the neoplastic cell to be properly restrained in the G_1 period.

The preceding section presents a general view of the composition of the cell life cycle. The sections to follow deal with certain aspects of the cell cycle with particular attention to possible mechanisms for regulation of cell reproduction.

The G_1 Phase

As already pointed out, the presence of the G_1 phase is not defined by any known events but is the time gap between the end of cell division and the initiation of DNA synthesis. Considerable information has, nevertheless, been obtained about its properties.

The existence of G_1 has sometimes been ascribed to preparations for DNA synthesis; in particular, the synthesis of enzymes needed to produce pools of the immediate precursors of DNA—i.e., the four deoxynucleoside triphosphates, and the synthesis of DNA polymerases and possibly other proteins concerned with configurational modifications of DNA in connection with replication. Some of these enzymes are, in certain cells, synthesized just before the initiation of DNA synthesis, an observation that supports the hypothesis already stated that a sequential cycle of gene activations underlies the cycle. But, at the very most, only the terminal part of G_1 can be explained by these enzyme syntheses, and the explanation for the existence of G_1 needs to be sought in other events.

Experiments with inhibitors have shown that both RNA and protein syntheses are necessary for a cell to progress through the G_1 phase, but this type of information is too nonspecific to afford insight into the reasons for G_1. Measurements of cell mass in mouse cells in culture have shown that the larger the cell at the beginning of G_1, the shorter the subsequent G_1 period.[3] The measurements demonstrate that the mass of the cell, or some component of cell mass, has an important bearing on the rate at which the G_1 events are accomplished, but unfortunately it does not contain any specific clue about the nature of these events. This relationship between cell mass and the length of G_1 may be interpreted to mean that G_1 is principally a period of cell growth, with the corollary that the mechanism that activates DNA synthesis is itself sensitive to total cytoplasmic mass or, more likely, to some specific part of cytoplasmic mass; for example, the amount of a particular protein. This important corollary is borne out by the observation in these experiments that in a population of cells there is less variation of mass among those cells initiating DNA synthesis than among those cells beginning the G_1 phase. This suggests that initiation of DNA synthesis is related to the attainment of a given cell mass or a given amount of some particular part of cell mass.

Any explanation of the G_1 phase must take into account the fact that a measurable G_1 phase can be absent in the life cycle of some cells. For example, ascites tumor cells in mice have no G_1 period under certain conditions permitting rapid growth,[4] and rapidly proliferating stem line cells of erythropoieses may have little or no G_1 phase. Cells in early cleavage stages in at least some animals lack G_1, although G_1 becomes a typical part of the life cycle for almost all cells of the embryo at later stages of development. Finally, a line of hamster cells has been described that grows without a G_1 period.[5] This is a particularly interesting example because this hamster cell line was almost certainly derived by a basic (genetic?) change from a hamster cell in which G_1 was a regular feature. Perhaps it might be tentatively concluded that under conditions that favor very rapid cell proliferation, the cell cycle is shortened by reduction of the G_1 phase to an immeasurably brief interval, or eliminated altogether. Unfortunately, we have no solid clues as to the underlying cause of G_1 elimination.

Whatever the reason that G_1 is present under some conditions and not under others, the observations reveal the important fact that G_1 is expendable as a time period with respect to maintenance of continuity of the cell life cycle. In general, the facts are consistent with the proposition (developed below) that the G_1 period is brought about by the controlled interruption of the cell life cycle —an interruption by which regulation of cell reproduction is achieved.

The most striking property yet discovered for the G_1 phase is its variability in length within a population of cells of any given type. This was first clearly noted for clonal populations of cells proliferating in culture with a constant average cell cycle time. All cells show cell cycle times that are normally quite variable even though the average generation time for the population remains constant. The variability is due primarily to variation in the length of the G_1 phase with relatively little or no fluctuation ordinarily present in the lengths of the S, G_2 or D phases. The observation has been extended to situations in which the average generation time for cells of a uniform type can be lengthened by a shift to slightly less favorable culture conditions. In such cases the increased generation time is accounted for largely, if not entirely,

by an expansion of the G_1 phase with little or no increase in the durations of S, G_2 or D. This is not to say that increases in S, G_2 or D do not occur under some conditions, but the major change is always in the length of G_1. An extreme extension of G_1 occurs in cells in culture that cease proliferation during stationary phase of culture growth; all the cells under stationary phase conditions are arrested in the G_1 state.

Especially pertinent is the arrest in G_1 of mammalian cells in culture as a consequence of contact inhibition. It is a well-established observation that normal (diploid) mammalian cells in culture cease migration and cell division when the cells become so crowded that large areas of contact between cells are established.

Regulation of Cell Reproduction

During cleavage stages of development, cell reproduction is extremely rapid. During this period of minimum restraint on cell reproduction, the cell life cycles are foreshortened by the elimination of any measureable G_1 period. At a point in the development of the embryo not yet determined, the rate of cell reproduction begins to decline in various groups of cells that have entered very early differentiation; there is some evidence that the reduction in reproductive rate is achieved by the introduction of a G_1 phase into the cells cycles. Finally, in the adult organism the degree of restraint operating on cell proliferation becomes stabilized, although it differs enormously from one tissue to another in a way that conforms precisely to the need for new cells to replace those lost by death. The degree of restraint in each tissue is exactly reflected in the time that the average cell requires to complete one life cycle. Average life cycle times, therefore, vary from many thousands of hours—in a very slowly renewing tissue such as liver—to 12 or less hours in stem line cells serving the very rapid turnover of erythrocytes. The normal demand for new erythrocytes in man is roughly 2.5 million per second, which means the completion and initiation of 2.5 million cell life cycles every second.

The dynamic state of the regulatory mechanism that maintains the different and appropriate rates of cell reproduction in each adult tissue is dramatically apparent in the changes in the rates of cell reproduction brought about by wounding of epithelia, partial hepatectomy, acute loss of blood cells, or similar disturbances to renewable tissues. The rate of cell proliferation is greatly accelerated in such cases of acute cell loss until the cell population is restored to the normal level.

The analysis of the life cycles for cells in a variety of renewing tissues has produced an important insight into the normal regulatory mechanism. For example, in the alimentary tract of the mouse, the average cell cycle time for the epithelial stem cells varies from 17 hours in the ileum to 36 hours in the colon, to 85 hours for buccal mucosa, to 181 hours in the esophagus.[6] It is a striking fact that these differences in cell cycle times are due to a change in the duration of only the G_1 period of the cycle. The combined duration of the S, G_2, and D phases is approximately 10 hours in all cases, while the duration of G_1 varies from approximately seven hours in the ileum, 25 hours in the colon, 75 hours for buccal mucosa, to 171 hours in the esophagus. Under some circumstances, such as suboptimal nutrient conditions (for example, because of a poor blood supply), the lengths of S, G_2 and D may be increased. But it is clear that the regulation of the rate at which tissue cells are allowed to reproduce is exercised by the retention of cells in the G_1 state for a shorter or longer period. Cell-cycle analyses are now available for the cells in a number of normal renewing tissues as well as in neoplasms, and they

bear out the generalization. Finally, in nonrenewing tissues of the adult (e.g., skeletal muscle cells and neurons) the differentiated cells are permanently arrested in the G_1 state.

This role of G_1 in the regulation of cell reproduction in renewing tissues and neoplasms is in striking accord with the established fact that in free-living cells of all types and mammalian cells grown in culture the natural arrest point for cell reproduction is in the G_1 phase. On this basis, it is reasonable to postulate that the regulation of cell reproduction in multicellular organisms has evolved out of the mechanisms by which cell reproduction is curtailed in free-living cells under natural (but unfavorable) conditions.

The discovery of this property of the G_1 phase represents a major and most significant advance in our understanding of cell reproduction and has brought into much sharper focus the whole problem of the regulation of cell reproduction and, most important, the loss of such regulation in neoplasia.

In this regard, it is pertinent to bring up once more the phenomenon of contact inhibition. We have very little idea of why or how contact between cells prevents their entrance into chromosome replication, but it is a most impressive fact that these cells can be released from inhibition by transformation with certain RNA or DNA viruses (e.g., polyoma virus, simian virus 40, Rous sarcoma virus).[7] Viral transformation of diploid, normal cells is measured as a release from contact inhibition of both migration and reproduction. One of the first signs that transformation has occurred is the exodus of cells from G_1 with the initiation of DNA synthesis. Shortly following the initial events of viral transformation, the cells undergo a pronounced karyotype change from diploid to aneuploid.

The changes that occur in transformation (i.e., release from inhibition of migration and reproduction and development of aneuploidy) parallel strikingly the changes that convert a normal cell into a neoplastic cell. Because transformation can be induced not only by viruses but by radiation or carcinogenic chemicals, and because at least some cells transformed in culture are capable of producing tumors when injected into animals, it can hardly be doubted that cell transformation in culture is essentially the same process by which cancer develops in vivo.

The breakdown in the control of cell proliferation must, as a corollary to the discovery of the G_1 arrest, or G_1 extension of the cell cycle, stem from a loss of sensitivity in one or more G_1 events to a tissue-specific regulator substance (co-repressor). (Fig. 2.) Such regulator substances are still undefined in spite of a hard search, but a variety of observations on normal tissue development, experiments on partial hepatectomy, unilateral nephrectomy and skin regeneration, and other studies all compel us to believe that they exist. Regulator substances are usually discussed in the context of the autoregulation hypothesis, which is composed of the following elements. It is clear that the differentiation of any cell type for performance of a particular, specialized function (for example, hepatocytes) at the same time includes the differentiation of a highly characteristic regulation of the reproductive rate for that given cell type. Quite obviously, differentiation of a fully functional hepatocyte can only be successful if this is accompanied by differentiation of the appropriate rate of cell reproduction (i.e., a rate sufficient to replace liver cells lost by death). If this differentiation of reproductive rate is incomplete or otherwise defective, a hepatoma is the result.

The differentiation of regulation over the rate of cell reproduction also includes a flexibility that allows the cell to accelerate greatly its rate of reproduction in response to the acute loss of

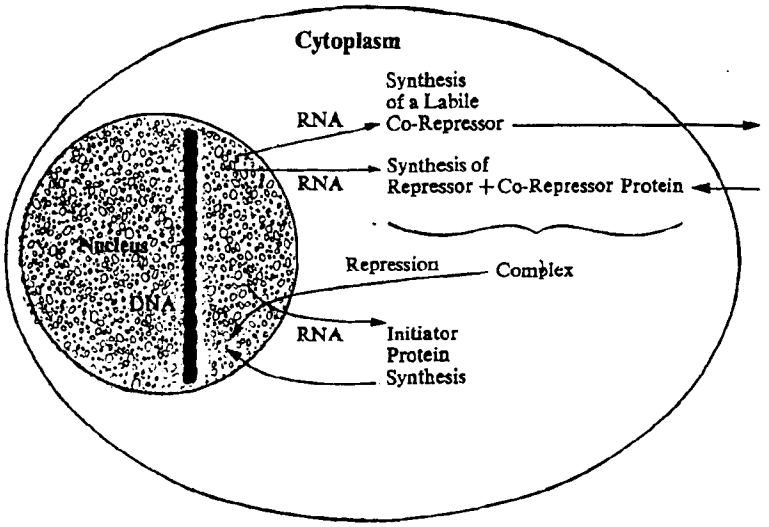

Fig. 2. A scheme for the regulation of reproduction of tissue cells. The scheme encompasses and connects the autoregulation hypothesis and the initiator protein concept. (See text for details). The cell restricts its own reproduction by synthesizing factors (co-repressor and repressor in this figure) that work together to prevent the synthesis of the RNA responsible for production of initiator proteins. The inhibition of the synthesis of initiator proteins prevents the initiation of DNA replication.

other cells of the same type in any renewable tissues (e.g., the increase in reproduction of hepatocytes in response to partial hepatectomy). Therefore, it has been postulated (Fig. 2) that, as a part of differentiation, each particular cell type establishes and maintains a specific microenvironment for itself by continuous addition to the microenvironment of a short-lived co-repressor that specifically interacts, by feedback, to inhibit cell reproduction in that particular cell type which produced the particular co-repressor. The production of the postulated co-repressor not only must be tissue specific but also must be tied in some fashion to the functional demands placed on the particular cell type in order to explain the capacity of renewing tissues to undergo hyperplasia or regression in response to a variable functional demand placed on the tissue. The co-repressor must also be liable or actively broken down if the hypothesis is to explain the dynamic response following such cell losses as partial hepatectomy.

The essence of the hypothesis must certainly be correct, although a regulator substance (co-repressor) has never been identified. What is important here is that

autoregulation, whatever its molecular mechanisms, works by holding cells in the G_1 phase of the cell cycle, and breakdown in a link of the autoregulatory mechanism results in neoplasia by allowing the entrance of cells into DNA synthesis. Without knowing any of the molecular details in autoregulation, we can hardly speculate about the specific details of their breakdown. Nevertheless, there are important indirect clues.

It is well established that a change in chromosomal constitution is a general accompaniment of the development of neoplasia whether the initiating stimulus is chemical, viral, radiation or unidentified (spontaneous). The karyotype changes are usually extensive and variable from neoplasm to neoplasm, and it has not been possible (with one or two exceptions) to associate any regular pattern of change with any particular neoplasm. It has been argued that the gross karyotype changes are secondary to the transformation of a cell to a neoplastic state and that the initial primary change is more subtle. It is, in fact, difficult to understand why gross chromosomal changes would be necessary to produce the G_1 phase breakdown in control of cell reproduction. A human tumor cell line with an apparently normal diploid karyotype has been reported.[8] In a few neoplasms the chromosome change that accompanies transformation is indeed small (e.g., the loss of a small piece of chromosome 21, the Philadelphia chromosome, present in chronic myelogenous leukemia). It seems significant that the loss of part of chromosome 21 in chronic myelogenous leukemia is detectable in normoblasts and megakaryocytes but is only effective in producing neoplasia in leukocytes. The observation is in keeping with the hypothesis that the breakdown in the regulation of cell reproduction is an intimate part of the differentiation of the particular cell type (in this case, leukocytes), and, therefore, transformation of different cell types (in this case, normoblasts and megakaryocytes) requires changes in different parts of the genetic apparatus.

In those cases of chronic myelogenous leukemia that lack any detectable loss from chromosome 21, it can be argued that the crucial deletion underlying this disease occurs at the very end of the chromosome, and that the deletion is therefore accomplished whether a large or undetectably small deletion is involved. The same argument applies to the apparently normal diploid human tumor mentioned in the paragraph above.

This argument on the minuteness of the chromosomal change necessary to produce neoplasia gains validity from the important discovery by Gateff and Schneiderman[9] that the development of an invasive, malignant, transplantable neuroblastoma in *Drosophila* larvae is due directly to the homozygous presence of a recessive mutation in the left tip of the second chromosome (with no other chromosomal change and certainly no change in karyotype). This clear evidence of a single mutational basis for a neoplasm is remindful of the unrecognized work of Gordon[10] many years ago proving beyond doubt the genetic basis of melanoma induction in certain fish. The fact that the neoplastic growth occurs only in neural tissue in *Drosophila* larvae even though the mutation is present in all tissues, and that the neoplastic growth occurs only in melanocytes in fish even though the same genetic makeup is present in all tissues, strongly indicates that transformation to the cancerous state is connected to a failure of the particular cell type to differentiate the normal regulatory mechanism that is unique to the differentiation process of that cell type. Obviously, it would be important for our understanding of cancer if the activi-

ty specified by the mutant locus in *Drosophila* could be identified. There appears to be, at present, no feasible way of making such an identification.

The fact that all neoplasms develop by a change in some G_1 event focuses special attention on this part of the cell cycle. Of particular importance is the transition from the G_1 into the S phase, because the initiation of DNA synthesis is the earliest identifiable point at which it is clear that the cell has become committed to another cell division. The transformation into a neoplastic state is first detected as the acceleration of entrance of cells into DNA synthesis. The breakdown in regulation of the initiation of DNA synthesis is therefore currently the most specific event by which we are able to explain the breakdown of regulation of cell reproduction.

A lesion in the control mechanism for DNA synthesis obviously is the principal issue in the development of neoplasia, but identification of the lesion seems very unlikely before the fully intact control mechanism is elucidated. Most of what is known about the control of DNA synthesis comes from the extensive and ingenious studies of microorganisms. It has been established that initiation of synthesis is brought about by the synthesis of a protein that apparently does not constitute any of the enzymes known to be necessary for DNA synthesis but instead acts as an initiator of DNA synthesis. What controls the synthesis of this initiator protein is one of several key questions. Mutants have been obtained in microorganisms in which the ability to initiate DNA synthesis has been lost, with some evidence that the alteration or loss of the initiator protein is the basis for the defect in initiation of DNA replication. Such information and concepts developed for microorganisms are beginning to have a profound impact on efforts to understand the control of DNA synthesis in higher cells, and it is likely that the development of new approaches to basic research on cancer will be guided more and more by the discoveries from microbial studies.

Figure 2 contains a speculative diagram of a tissue cell that attempts to encompass and connect, with the least number of postulated elements as possible, both the autoregulation idea and the initiator protein hypothesis. Initiation of DNA synthesis is postulated to be brought about by an initiator protein, the synthesis of which is controlled at the transcription level (messenger RNA synthesis). The synthesis of this messenger RNA is repressed by the complex between a repressor protein and a co-repressor. Both the repressor protein and the co-repressor are synthesized continuously, and both are specific for any given cell type; the repressor protein remains intracellular while the labile co-repressor equilibrates with the space of the microenvironment. As long as there is sufficient co-repressor in the system, DNA synthesis is repressed. If the effective concentration of the labile co-repressor in the microenvironment is reduced by acute loss of cells, or is destroyed at an increased rate as a consequence of increased functional demand on the particular cell type, repression of DNA synthesis is weakened in some proportion to the decrease in the amount of repressor-co-repressor complex. The active destruction of co-repressor at a rate dependent on the functional load is, in this scheme, proposed as the means by which functional load is communicated to the regulation of DNA synthesis and cell reproduction.

Without presenting the details of the argument, it seems most likely that the breakdown in the mechanism that controls DNA synthesis most likely would occur in the regulation of synthesis of repressor protein, or in a

mutation of the repressor protein gene itself. The development of the proper amount of regulation may be considered a part of the total pattern of differentiation of that cell type; the less the differentiation of the cell, the less complete the development of regulation of repressor synthesis. Inclusion of the property of specificity by cell type for both co-repressor and repressor is sufficient to explain independent regulation from one tissue cell type to the next and is particularly pertinent to the fact that neoplasia are highly tissue specific.

All these hypothetical arrangements may yield a picture that seems just too contrived and fanciful in view of the dearth of solid information, but most of this scheme has a counterpart in the mechanism of regulation of RNA transcription in microorganisms that is based on firm fact. Elements of the diagram (Fig. 2) have already been published by others.

In any case, the elucidation of the molecular mechanisms that control the initiation of DNA synthesis will put within much closer reach a full explanation of the regulation and the loss of regulation of cell reproduction in mammalian tissues. At this point it is quite clear that the problem of loss of regulation of cell reproduction in neoplasia is precisely a question of a genetically based loss of control over initiation of DNA replication, and until we solve these pointed problems, the chance of finding a prevention or cure to cancer is extremely remote.

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A Spirit of Adventure: A Personal Tribute to Wendell G. Scott, M.D. (1905-1972)

The onrushing flood of future possibilities brings with them a spirit of adventure: a need for bold, imaginative thinking; a willingness to gamble on provocative new concepts; the courage to break away from entrenched viewpoints, and the aggressiveness to bring them into reality.

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It was exhilarating just being in his presence. His quick smile and spontaneous chuckle induced an atmosphere of friendship and warmth. His bright and alert look, the way he leaned forward to greet you, said that here was a man of action. He was impatient, although always courteous and diplomatic, about unnecessary delays, postponements and obstacles to rapid achievement, and a meticulous planner of the details and organization needed to get the desired results. This was Dr. Wendell G. Scott —a man of extreme capability and considerable achievement.

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Arthur I. Holleb M.D.

In hospitals, as in all other health facilities, what really mattered, above any kind of technical consideration, was political relevance.

From this brief review of the effects of social revolution on the public health of Chile, a basic message emerges. The health status of a developing nation depends upon an equilibrium between economic, social and political factors but with due regard for scientific and technical advances. A solely political or ideologic approach to health and medical problems cannot solve the low standard of health of these populations. Only if the technical and scientific levels of medicine are increased in concert with a balanced and progressive social change, is it possible to close the large gap between the health levels of Western democracies and those of the developing countries.

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MEDICAL PROGRESS

THE CANCER CELL: DYNAMIC ASPECTS AND MODIFICATIONS IN CELL-SURFACE ORGANIZATION (~~First of Two Parts~~)

GARTH L. NICOLSON, PH.D., AND GEORGE POSTE, M.D., PH.D.

THE essential features of tumor cells that distinguish them from their normal counterparts are their ability to proliferate in an uncontrolled fashion, invade normal tissues and metastasize to distant sites, though these behavioral alterations are not always coupled. It is now recognized that changes in the surface properties of tumor cells may be important in determining aspects of each of these major behavioral characteristics.

Within the primary tumor, alterations in the surface properties of tumor cells contribute to their escape from many of the controls and social restraints to which normal cells are subject. The proliferation of tumor cells is no longer effectively regulated by cell-to-cell contact interactions, and they are increasingly unresponsive to growth regulation by serum factors, hormones and other agents that exert their effects after binding to the cell surface. Tumor cells can therefore achieve varying degrees of autonomy from normal growth restraints, and this ability is reflected in uncontrolled proliferation and progressive enlargement of the primary tumor. Altered surface properties of tumor cells may also result in aberrant cell-to-cell recognition, allowing tumor cells to escape from the control mechanisms responsible for maintaining proper cell position. In malignant lesions these processes are augmented by metastasis, in which the surface properties of tumor cells are important not only in the invasion of surrounding normal tissue (or tissues) and initial separation of cells from the primary tumor, but also in determining the subsequent pattern (or patterns) of cell distribution and establishment of metastatic foci. Finally, the outcome of the interaction of tumor cells with elements of the host immune apparatus in both primary and secondary tumors is influenced in large part by the surface properties of the tumor-cell population.

To understand these complex phenomena, the properties of cell membranes and the surface organization of normal and neoplastic cells must be comprehended. Over the last decade knowledge of the

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structure and dynamic organization of cellular membranes has increased substantially, and a large number of surface differences between tumor cells and their normal counterparts have been identified. A full discussion of this vast subject is impossible within the limits of the present article. We will instead restrict our comments to a brief review of recent data obtained in several laboratories suggesting that the topographic rearrangement of various cell-surface components may be important in determining the expression of certain of the altered surface properties and aberrant behavioral characteristics displayed by tumor cells. Particular emphasis will be given to the control of cell-surface receptors by cytoskeletal elements situated in the cytoplasm and the related question of how changes in these trans-membrane control systems[1] might account for some of the surface modifications associated with neoplasia.[2]

Cell-Surface Organization

Cell membranes are composed of lipids, proteins, oligosaccharides and polysaccharides.[3] The most likely arrangement for these components is one that maximizes the lowest free energy environments for each constituent. For lipids (such as phospholipids), which are structurally asymmetric in the hydrophilic and hydrophobic portions of their structures, hydrocarbon tails in solution tend to associate and exclude water whereas ionic heads tend to seek interactions in the aqueous phase. These interactions favor the formation of a continuous bilayer configuration for membrane lipids that sequesters lipid hydrocarbon tails from the aqueous phase.[3,4] The lipid molecules on each side of the bilayer are also arranged asymmetrically in at least certain cell membranes,[5] and they are capable of rapid lateral motion in the plane of the bilayer giving most biologic membranes the viscosity of light oil.[1,3,4,6] Lipids can also segregate in membranes to form specific domains with different average compositions, and they can associate with membrane proteins ("boundary lipid") to form lipoprotein complexes.[1] In addition, some membrane lipid may exist in a "frozen" gel state segregated from the bulk fluid lipid.[4] These various lipid states may be important in organizing cell membranes into specific domains, although available evidence[1,3,4-6] suggests that most lipid molecules are in a fluid state and are freely diffusable in the membrane plane.

Membrane proteins are basically of two types: those stabilized in the membrane by hydrophobic interactions (for example, components bound by water exclusion and interaction with lipid hydrocarbon tails); and those stabilized onto the membrane surface by hydrophilic interactions (for example, components surrounded by aqueous environment and charged groups and bound to the membrane by other than hydrophobic forces). Proteins in the former group have been termed integral membrane proteins,[3] and this class of proteins appears to be involved in phenomena such as metabolite and ion transport.[1,3] Integral membrane proteins (and glycoproteins) are globular, bimodal proteins that interact in both the hydrophobic (hydrocarbon) and the hydrophilic parts of the membrane.[1-3] Thus, they are intercalated into the fluid lipid bilayer to various depths, depending on their amino acid sequences and three-dimensional folding (Fig. 1). It is important that some integral proteins and glycoproteins can completely span the membrane[1,3,5] and have hydrophilic peptide sequences on

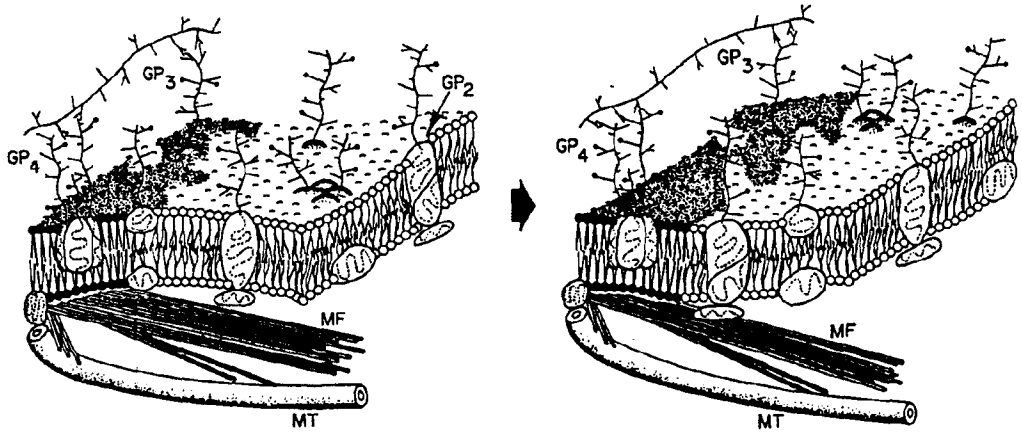

Figure 1. Hypothetical Trans-membrane Control over the Distribution and Mobility of Cell-Surface Receptors by Peripheral and Membrane-Associated Cytoskeletal Components (Reproduced from Nicolson[1] with the Permission of the Publisher).
In this schematic model of membrane organization the mobility of integral glycoprotein complexes GP_3 and GP_4 is controlled by outer-surface peripheral components and also trans-membrane linkages to membrane-associated cytoskeletal elements. In addition, complexes GP_3 and GP_4 could be sequestered into a specific lipid domain indicated by the shaded area, whereas complex GP_2 exists in a free or "unanchored" state and is capable of lateral motion in the membrane plane formed by the fluid lipid matrix. MT denotes microtubules, and MF microfilaments (not to scale).

both the external and the cytoplasmic membrane faces (Fig. 1).

The second group of proteins, termed peripheral membrane proteins,[3] seem to be involved in a variety of roles not essential to the basic structure of biologic membranes. Peripheral membrane proteins usually associate with integral membrane proteins and glycolipids by ionic interactions that can be disrupted by high salt concentrations or chelating agents.[3] Removal of peripheral membrane components does not result in destruction or solubilization of the membranes, whereas removal of integral proteins is usually accompanied by dissolution of membrane structure.[3]

The cell membrane in its most simple form can be considered a two-dimensional solution of a mosaic of integral membrane proteins embedded in a fluid lipid bilayer.[3] This concept of membrane architecture has two important implications for membrane function. In the first place, the arrangement permits membrane components to be organized asymmetrically, thus allowing certain membrane components to be localized predominantly in either the outer or the inner half of the membrane.[3,5] Membrane glycoproteins and glycolipids, for example, are arranged so that their carbohydrate residues are exposed exclusively at the outer face of the cell membrane (Fig. 1), where they function as specific receptors for antibodies, hormones, lectins, viruses and other agents.[1,3] Maintenance of membrane asymmetry dictates that components do not continually rotate through the membrane from one side to the other. Restriction of such trans-membrane rotation (or so called flip-flop) has recently been demonstrated experimentally.[6]

The second important feature of the type of membrane organization is that components can diffuse laterally within the plane of the membrane.[1,3,4-6] This diffusion offers a potential mechanism for rapid and reversible changes in the topography of specific membrane components (Fig. 1). Various integral proteins and lipids have been shown to move laterally within the membrane, though at quite different rates.[4] The lateral diffusion of lipids is rapid, each molecule exchanging with its neighbor about 10^7 times a second.[6] A number of integral membrane proteins and glycoproteins are also able to diffuse laterally within the membrane but at much slower rates than the lipids,[1,2,4,7-9] whereas certain proteins appear to be relatively immobile or "anchored" within the membrane.[1,8-10] The distinct mobilities of unique classes of integral proteins and glycoproteins within the plasma membrane suggest that the cell may possess a control mechanism that restricts the mobility of some components to maintain ordered topographic displays or "patterns" of molecules on the cell surface.[10] Such patterns might well be highly specific, differing on individual cell types and specific regions of the same cells, and they might also change during various stages of cellular activity.

Topographic molecular patterns could be fundamental to the inherent specificity of cell-surface organization. The existence of cell-specific surface patterns could constitute an important mechanism for determining cell contact and recognition phenomena and for transmission of cell positional information in tissues. In addition, topographic redistribution of surface components might occur in response to various environmental stimuli, offering a mechanism for rapid and reversible modulation of cell-surface properties after interaction of such agents as mitogens, serum factors, hormones, lectins and antibodies with the cell surface.

There is an increasing amount of evidence that the translational mobility of certain classes of integral membrane proteins is controlled by cytoplasmic skeletal elements composed of microtubules and microfilaments that appear to be "linked" in some way to integral membrane proteins.[1,2,7,8] This concept has been termed trans-membrane cytoskeletal control[1] because any regulatory influence exerted by cytoskeletal elements on the mobility of integral membrane components will simultaneously affect components on the outer face of the membrane that are trans-membrane linked to this cytoskeletal system (Fig. 1).

Microtubules are large, rigid tubular structures with an outside diameter of 25 nm and an inner core 15 nm in diameter. These structures, which are constructed predominantly from a protein subunit called tubulin, have been found in both the nucleus and the cytoplasm (in the latter often associated with the cell membrane) in a wide variety of cells.[11] Cytoplasmic microtubules appear to undergo rapid and reversible assembly and disassembly, and although control of these processes is unclear, calcium ions and cyclic nucleotides appear to be involved.[11] Microtubules can be induced to dissociate by pressure and low temperatures, and their assembly is inhibited by plant alkaloids such as colchicine and vinblastine.[11]

Microtubules are often found in association with another class of cytoskeletal elements, the microfilaments. The latter are thin (approximately 6 to 8 nm in diameter), double helical filamentous structures that are frequently associated with the plasma membrane in bundles or sheets immediately apposed to the inner face of the membrane.[12] The identification of actin, myosin and tropomyosin in association with microfilament bundles[12,13] has contributed to the belief that they are contractile and perform important muscle-like functions in cell locomotion, cell-shape changes, cytoplasmic streaming and the movement of various plasma membrane components (see below).

Although microtubules and microfilaments are not true cell-membrane components, their control of the mobility and distribution of certain cell-surface receptors indicates that they are in some way trans-membrane "linked" to surface components, and this characteristic justifies their classification as membrane-associated components.[1]

Trans-membrane Cytoskeletal Control of Surface Receptors

The binding of multivalent ligands to receptors on the cell surface can either cause the receptors to remain relatively dispersed or produce movement of receptor-ligand complexes into groups or "clusters" (Fig. 2). In some cases the clusters migrate into much larger "patches," and on some cells the patches eventually form "caps" (Fig. 2). Alternatively, the clusters coalesce into specific regions, where the receptor-ligand complexes are in time internalized by endocytosis.[1,2,14,15] Examples of multivalent ligands showing these properties are antibodies[1,14,15] and lectins[1,7,16] (proteins and glycoproteins that bind to specific classes of oligosaccharide sequences).[16]

The binding of antibodies directed against cell-surface immunoglobulin (Ig) receptors on lymphoid cells results in anti-Ig-induced capping followed by endocytosis or "shedding" of the Ig-receptor-antibody complexes.[1,14,15] Capping of surface-Ig receptors can be inhibited or even reversed by drugs that act on the membrane-associated cytoskeletal system such as cytochalasin B, a fungal metabolite that disrupts microfilaments. Similarly, inhibition of cellular metabolic machinery also blocks cap formation.[1,14] These results suggest that microfilaments play an active part in ligand-induced capping and are responsible for translocation of the receptor-ligand clusters and patches into caps. The binding of lectins such as concanavalin A (Con A), which recognizes α-D-mannosyl-like residues in oligosaccharides, to the surface of lymphocytes inhibits subsequent capping of surface-Ig by anti-Ig.[7,8] The inhibition of anti-Ig-induced capping by Con A, however, can be overcome by drugs such as colchicine or vinblastine that impair microtubule function. Nevertheless, if Con A is bound to lymphocytes at low temperature (0 to 5°C), it can induce capping of its own receptors.[7,8] The interpretation of these apparently contradictory results is that both Con A receptors and surface-Ig receptors are linked through a microtubule system that "anchors" these receptors. In the presence of microtubule-disrupting drugs or under conditions promoting microtubule depolymerization (such as low temperature), the anchorage is broken, and anti-Ig, as well as Con A, induces cap formation.[7,8] Not all lymphoid cells can be easily capped with Con A, but in some of these cases, addition of colchicine dramatically facilitates capping.[2]

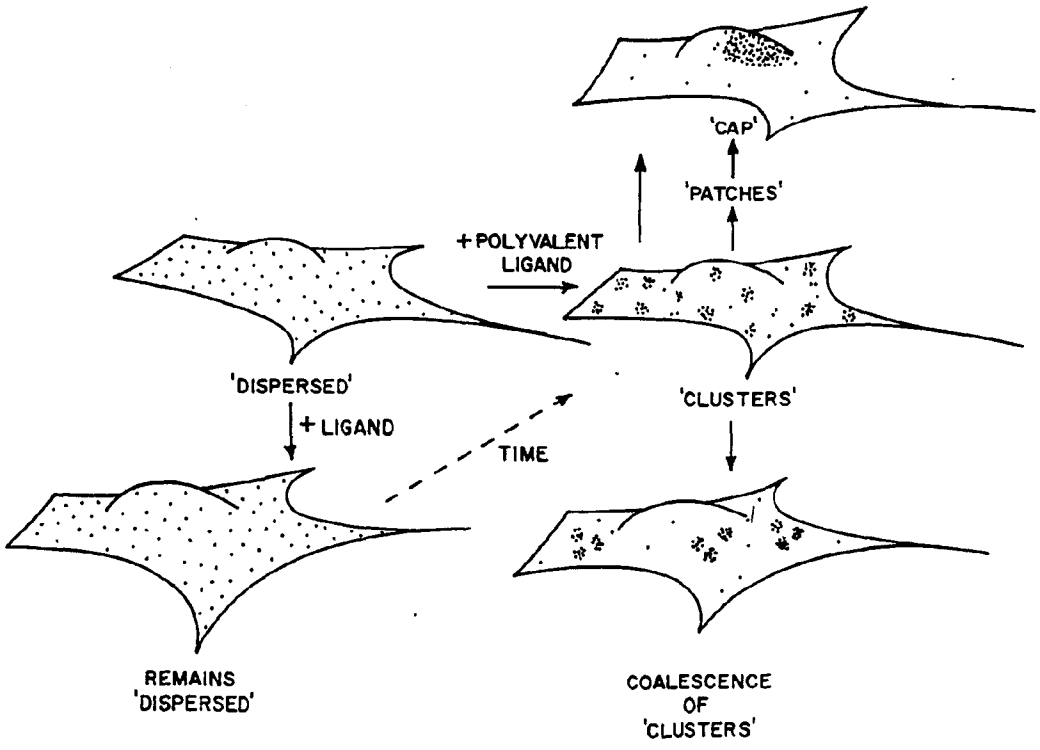

Figure 2. Alternate Pathways of Ligand-Induced Receptor Redistribution on Fibroblastic Cells (Reproduced from Nicolson[1] with the Permission of the Publisher).

After ligand binding, initially dispersed receptors can remain dispersed or undergo ligand-induced clustering. The clustered receptor-ligand complexes can coalesce before endocytosis, or they can form "patches" and eventually "caps".

Non-random movements of cell-surface receptors into specific membrane regions under the influence of the cytoskeletal system has also been documented during phagocytosis in polymorphonuclear leukocytes.[2] Phagocytosis of large particles such as polyvinyltoluene spheres by these cells results in concomitant loss of lectin receptors from the cell surface. Since the receptors are present initially in a random distribution, the loss of surface lectin receptors should parallel the loss in cell-surface area after phagocytosis. Normally, this action does not occur, and a larger number of lectin receptors are removed than expected from the relative differences in surface areas. However, if colchicine is present during phagocytosis, selective loss of lectin receptors from the cell surface is prevented.[2]

Further insight into the role of membrane-associated cytoskeletal elements in controlling the distribution and mobility of a number of integral plasma membrane proteins has come from recent studies using tertiary amine local anesthetics. Until recently, these drugs were believed to interact exclusively with membrane lipids, but evidence obtained within the past two years in several laboratories has shown that they also affect intracellular microtubules and microfilaments.[17,18]

Tertiary amine anesthetics of the cocaine series (dibucaine, tetracaine, procaine, etc.) dramatically enhance lectin agglutinability and lectin-induced receptor redistribution on fibroblastic cells, but inhibit lectin and antibody-induced capping on lymphoid cells.[17,18] Local anesthetics are known to partition into the fluid lipid bilayer, where they associate with acidic phospholipids. At high concentrations local anesthetics are known to enhance the fluidity of membrane lipids.[19] However, at the concentrations active in producing a variety of effects on the dynamics of cell-surface receptors,[17,18] local anesthetics have little effect on cell-membrane fluidity.[17] The site of action of local anesthetics in modifying the dynamics and trans-membrane control of surface receptors is now thought to be on the membrane-associated cytoskeletal system for the following reasons: agglutination of untransformed mouse fibroblastic cells by lectins does not occur at low lectin concentrations where a relatively low rate of lectin-induced redistribution occurs,[16] unless the cells are first treated with local anesthetics, which enhance lectin-induced agglutinability and receptor redistribution[17,18]; local anesthetics reverse the inhibition of Con-A-induced agglutination of transformed mouse fibroblastic cells by colchicine and vinblastine[17]; lymphoid cell capping is prevented by local anesthetics[17,18,20]; and local anesthetics reverse the assemblage of performed caps on lymphocytes.[18] The fact that the above properties of local anesthetic-treated cells can be duplicated by treatment with colchicine or vinblastine *together* with cytochalasin B (colchicine or cytochalasin B alone will not suffice) strongly suggests that colchicine-sensitive microtubules and cytochalasin-B-sensitive microfilaments are both involved in maintenance of surface-receptor distribution and dynamics.[1,17,18]

More direct evidence that local anesthetics modify cytoskeletal elements or their plasma membrane attachment points has been obtained with electron mi-

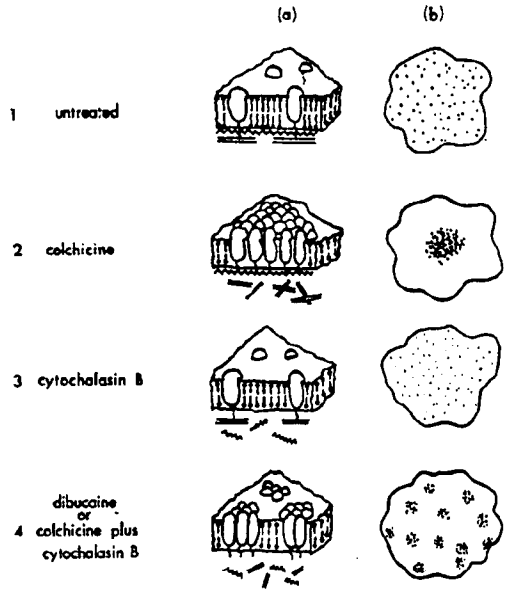

Figure 3. Schematic Representation of the Effect of Colchicine, Cytochalasin B and Dibucaine Hydrochloride on the Distribution of Concanavalin A Receptors on Untransformed Mouse 3T3 Fibroblastic Cells (Reproduced from Poste et Al.[17] with the Permission of the Publisher).

(a) denotes a segment of plasma membrane showing intrinsic membrane proteins carrying concanavalin A receptors embedded within a lipid bilayer and linked on the inner membrane face to microtubules (═══) and microfilaments (~~~). (b) is a representation of the planar view of the distribution of concanavalin A receptors over the upper surface of the entire cell. In 1, untreated 3T3 cells, (a) represents the integral proteins linked to both microtubules and microfilaments, and (b) concanavalin A receptors remaining randomly dispersed after binding of concanavalin A to the cell surface. In (2) 3T3 cells treated with colchicine, (a) shows that disruption of microtubules by colchicine allows concanavalin-A-induced aggregation of integral proteins that are still linked to an intact microfilament system, resulting in redistribution of concanavalin A receptors into a single large "cap" as shown in (b). In (3), 3T3 cells treated with cytochalasin B, (a) demonstrates that despite disruption of microfilaments by cytochalasin B, integral membrane proteins remain "anchored" by their connection to microtubules, and binding of concanavalin A to the cell surface does not induce redistribution of concanavalin A receptors, which remain randomly dispersed as shown in (b). In (4), 3T3 cells after treatment with dibucaine or combined treatment with colchicine and cytochalasin B, (a) indicates that disruption of the linkage of integral membrane proteins to both microtubules and microfilaments allows concanavalin-A-mediated redistribution of concanavalin A receptors to form multiple 'clusters' and 'patches' distributed over the entire cell as shown in (b).

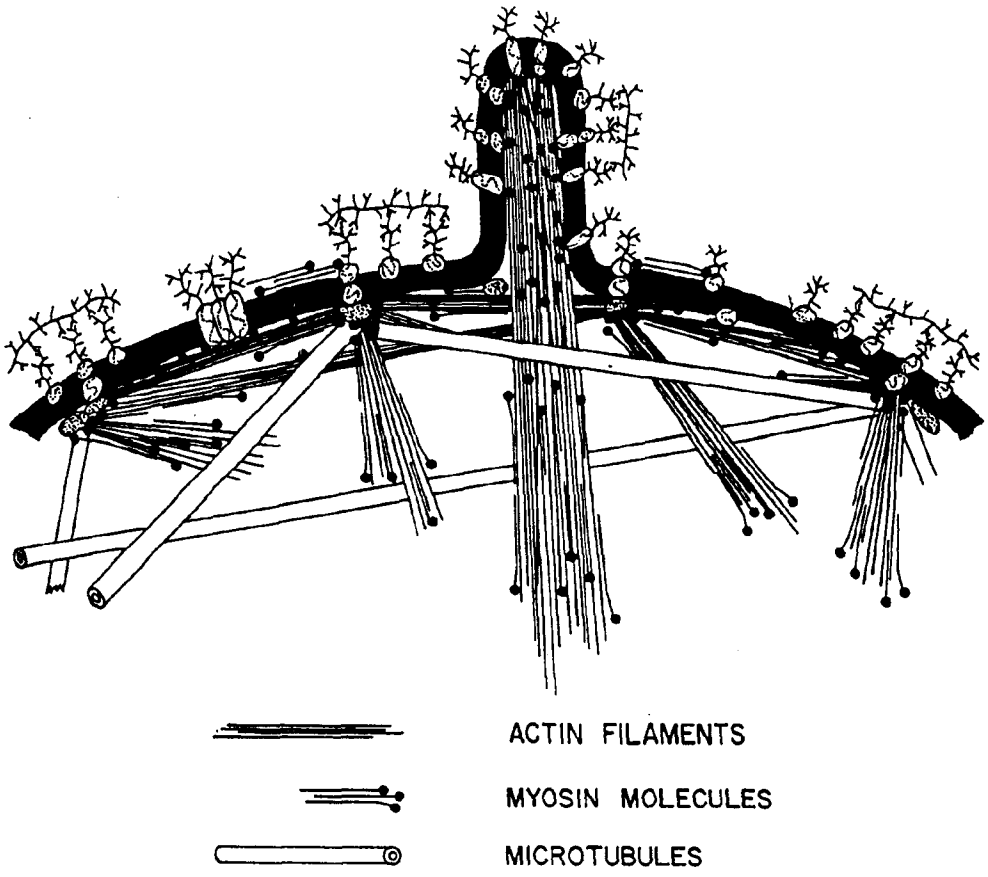

Figure 4. Hypothetical Interactions between Membrane-Associated Microtubule and Microfilament Systems Involved in Trans-membrane Control over Cell-Surface-Receptor Mobility and Distribution (Reproduced from Nicolson[1] with the Permission of the Publisher).

This schematic model envisages opposite roles for microfilaments (contractile) and microtubules (skeletal) and suggests that they are linked to one another or to the same plasma membrane inner surface components at specific "nucleation" points. In addition, peripheral membrane components at the inner and outer membrane surface may extend this control over specific membrane domains (not to scale).

croscopy. Concentrations of dibucaine, tetracaine or procaine that modify surface-receptor dynamics result in cell rounding and disappearance of cell-membrane-associated microfilaments and microtubules.[21] The mechanism of local anesthetic action in disrupting cytoskeletal systems is unclear, but they could increase intracellular Ca^{2+} concentrations to levels sufficient to depolymerize microtubules by displacing membrane Ca^{2+}. The effects of local anesthetics on microfilament integrity are even less certain, but these drugs might affect Ca^{2+}-sensitive components such as actomyosin complexes, adenylcyclase or guanylcyclase, Ca^{2+}-requiring ATP-ases, or many other Ca^{2+}-dependent processes.[18,21]

The various effects of the different classes or combinations of drugs discussed above point to a system of trans-membrane control of receptor mobility in which receptors are linked to both microfilaments and microtubules and suggest that these two classes of cytoskeletal elements have opposing roles in controlling receptor mobility.[18] Specifically, the microtubule system is envisaged as acting as a system of anchoring elements that restrain or limit the mobility of certain receptors. In contrast, the microfilaments are believed to act as a contractile system to redistribute clusters of receptors unless they are restrained by the anchoring microtubules.

If, as suggested, certain surface receptors are trans-membrane linked to both cytoskeletal systems (Fig. 3.*1*), that action would offer a reasonable explanation for the following observations: disruption of microtubules by colchicine or vinblastine allows microfilament contraction and functional dislocation of surface receptors from anchoring components formerly

stabilized by microtubules — in this case membrane-associated microfilaments would redistribute the "unanchored" receptor-ligand clusters into caps (Fig. 3.2); and disruption of microfilaments by cytochalasin B allows ligand-induced clustering unless the receptors remain trans-membrane linked to a microtubule anchoring system — in this case the anchoring structure would be expected to prevent any redistribution of receptors and would remain randomly dispersed (Fig. 3.3). Destruction of both the microfilament and microtubule systems with either local anesthetics or a combination of colchicine and cytochalasin B will result in the formation of random multiple clusters (Fig. 3.4).

The synthesis of various results and theories on trans-membrane control mechanisms[1,2,7,17,18] has been incorporated into a unifying model that is consistent with the results discussed here and elsewhere in detail.[1,18] Specifically, microtubule and microfilament assemblages are proposed to be interconnected and coupled to similar trans-membrane anchorage points. These anchorage points could serve as regulatory centers for the organization of specific membrane domains. The contractile movements of the microfilament system would be opposed by an anchorage system made up of microtubules (Fig. 4),[1] and thus offer a system whereby the cell surface and its receptors would be under fine control by opposing membrane-associated cytoskeletal elements.[1,18]

Cell-surface modifications occurring after neoplastic transformation are discussed in Part 2 of this series.

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MEDICAL PROGRESS

THE CANCER CELL: DYNAMIC ASPECTS AND MODIFICATIONS IN CELL-SURFACE ORGANIZATION

(Second of Two Parts)

GARTH L. NICOLSON, PH.D., AND GEORGE POSTE, M.D., PH.D.

CELL-SURFACE MODIFICATIONS ON CANCER CELLS

The central importance of the cell surface in determining many features of tumor-cell behavior has served to stimulate a massive research effort to identify differences in the surface properties of normal cells and their neoplastic counterparts. This effort has been successful to the extent that an enormous catalogue of differences between normal and tumor-cell populations has been accumulated (Fig. 5).[22,23] We now have little insight, however, into the mechanisms by which these manifold changes in surface properties arise in tumor cells and how these changes are maintained. Of more immediate importance is the fact that we are still largely ignorant of how individual cell-surface alterations contribute to complex-multi-component phenotypic alterations such as tumorigenicity, invasiveness and metastasis.[22-24]

In experimental studies on cancer most investigators have chosen to use cloned, untransformed tissue-culture cell lines, which can be transformed by oncogenic viruses, chemical carcinogens, or radiation to form stable cultured cell lines that can be assayed in vivo for their tumorigenicity. An alternative method has been to remove tumors and surrounding normal tissues surgically for simultaneous study or to collect leukemic cells for comparison to normal lymphoid cell populations. These "in vivo" approaches are complicated by the fact that most tumor cells arise from unknown precursors, making comparisons with other cells difficult.[22-24] Because of these problems and the limited availability of uniform cell populations, the main tools of the cancer-cell biologist have thus been model systems employing untransformed/transformed tissue-culture cell lines, frequently of rodent or avian origin.

It should be stressed, however, that many features of cellular organization are greatly affected by cultivation of cells in vitro. Awareness of the phenotypic changes that can be imposed on cell populations by cultivation in vitro is therefore of paramount importance if such technics are to provide a meaningful experimental approach to the study of transformation and neoplasia.[25]

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Alterations in Surface Composition

There are a variety of molecules present at the surface of cells, such as glycoproteins, glycolipids, glycosaminoglycans and acid mucopolysaccharides. The latter compounds form the "glycocalix" or extracellular saccharide-containing zone. Few changes in glycocalix components seem to be of general importance in neoplasia,[23] but research in this area has not been as active as the study of changes in glycolipids and their association with neoplasia.

Modifications of glycolipid structure and content after transformation have been fully reviewed by Hakomori[26] and Brady and Fishman.[27] Two of the relatively general changes in glycolipid structure have been decreases in the more complex glycolipids and deletion of glycolipid terminal saccharide residues. A loss in ability of glycolipid synthesis to respond to cell contact, as well as an increase in accessibility of glycolipids to antibodies, lectins and enzymes, is also associated with neoplasia.[26,27] One recent observation of interest is that some spontaneous mouse and hamster transformed cell lines do not show the generalized glycolipid changes such as structural simplification that are characteristic of the transformed state.

Several studies have concentrated on changes in surface proteins detectable by lactoperoxidase-catalyzed iodination. With this technic cell-surface proteins are labeled in their tyrosine residues with ^{125}I (reviewed elsewhere[22,23,28]). After labeling, the ^{125}I-proteins are solubilized and separated by electrophoresis on polyacrylamide gels in the presence of detergent. The most prominent change in surface-protein exposure is the loss of a high-molecular-weight (210,000 to 250,000 daltons) glycoprotein after transformation. This glycoprotein has been called a variety of names (LETS, galactoprotein *a*, CSP, component Z, SF210).[23,28] Interest in this component subsided somewhat with the finding by Yamada and Weston[29] that it will not restore density-dependent inhibition of growth, a characteristic of normal cells in culture, when added back to transformed cells. However, the possibility exists that under these conditions, the glycoprotein would not associate with the plasma membrane in sufficient amounts or would not be oriented correctly on the cell surface. Alternatively, it could associate with the cell surface, but fail to establish "linkage" with cytoplasmic cytoskeletal elements.

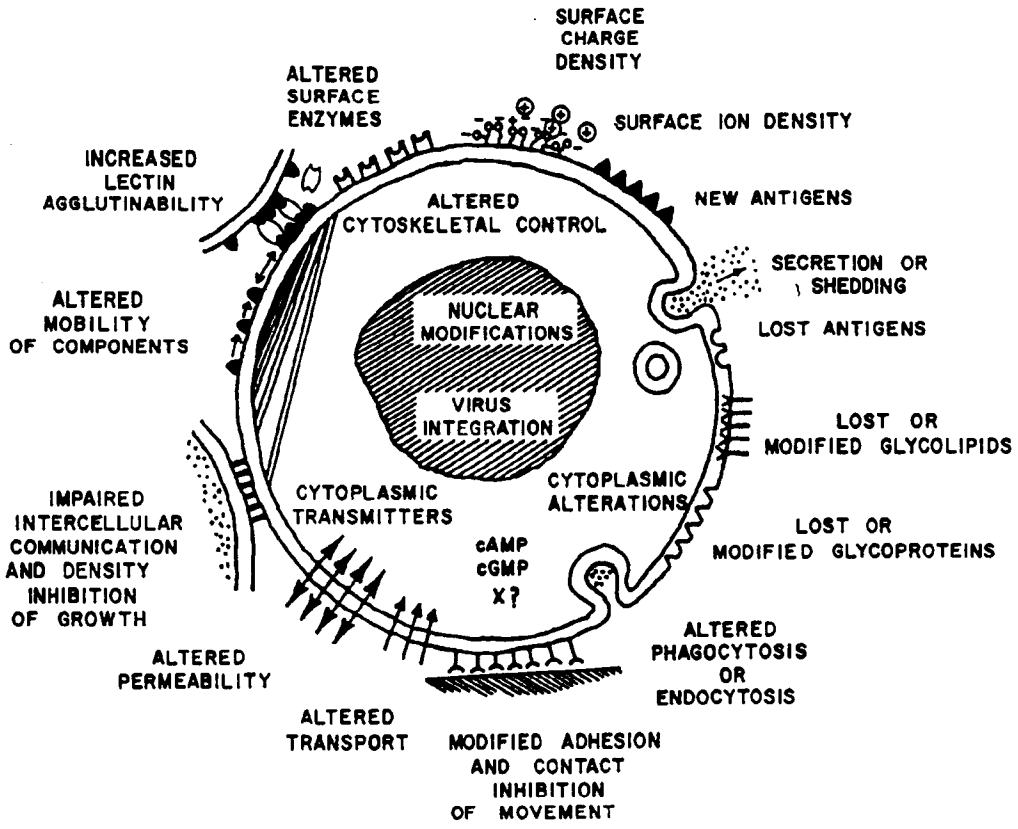

Figure 5. Some Cell-Surface Alterations Found after Neoplastic Transformation (Reproduced from Nicolson[23] with the Permission of the Publisher).

Fragments of proteins can be removed from the cell surface by proteolytic enzyme treatment and subsequently characterized in detail. Glycopeptides (average molecular weight of approximately 4000 daltons) removed from the surfaces of tumor and cultured transformed cells show transformation-related differences in elution profiles when the peptides are chromatographed on gel-filtration columns.[30] Specifically, the peptides from transformed and tumor cells are of higher apparent molecular weight, but their elution can be rendered similar to the normal glycopeptide elution profile by removal of sialic acid by the enzyme neuraminidase. The glycopeptides appear to be quite heterogeneous, and their characterization awaits further study. One problem with this approach will be the identification of the released glycopeptides for their proper molecular origin on the cell surface.

Alterations in Surface Enzymes

A variety of observations suggest that transformation results in increased levels of cell degradative enzymes such as proteases and glycosidases.[21-24,31] Unfortunately, many of these studies were performed without distinguishing between surface-localized, secreted or total enzyme levels. When pathological tissue samples are examined, little attempt is usually made to determine leukocyte count or other cell contamination. Hence, these observations are often not reliable.[23] In tissue-culture systems transformation generally increases the levels of specific glycosidases and proteases.[22,23]

Proteases have been implicated in loss of growth regulation after transformation,[23,24,28,31] and they are thought to be a prominent determinant of several of the surface properties characteristic of the transformed state such as alterations in lectin agglutinability,[16] mobility of surface components,[16,23] changes in sugar and phosphate transport, reactivity with antibodies and others.[22-24] When untransformed cells are treated with proteases, they transiently acquire many of the properties routinely possessed by transformed cells.[23,24] Holley[32] has recently proposed that protease treatment may mimic hormonal stimulation of growth by acting on cell-surface hormone receptors. Additional circumstantial support for the view that proteolytic modification of the cell surface might account for certain altered surface properties found in tumor cells has come from a large body of experimental work showing that the abnormal growth properties demonstrated by transformed cells in vitro and some of their altered surface properties can be modified by protease inhibitors.[23,24,31]

Alterations in Transport and Cyclic Nucleotides

Transformed cells generally have higher plasma-membrane transport activities than their untransformed counterparts.[22,23] Transport of several, but not all, sugars is enhanced by neoplastic transformation,[33] and transformed cells show greater rates of uptake of certain amino acids and amino acid analogues.[22,23] Interestingly enough, many of these transport modifications can be mimicked in untransformed cells by treatment with proteases, glycosidases, serum or hormones.[23] Serum addition can release quiescent untransformed cells from density-dependent inhibition and also stimulate sugar, nucleotide, and phosphate transport.[32]

Concurrent with some of these transport changes are rapid increases in a cellular cyclic nucleotide, cyclic GMP.[34] The levels of cellular cyclic nucleotides have been proposed to be the determining factor (or factors) in releasing quiescent normal cells from growth regulation and permanently maintaining transformed cells in a state of unrestrained growth.[34,35] Cyclic nucleotides may serve as intracellular messages that alter cytoplasmic enzymes, transport systems and perhaps more directly regulate DNA synthesis.[34,35]

Alterations in Receptor Mobility

There are several observations indicating that trans-membrane control over cell-surface receptors is altered by transformation. Modifications in lectin agglutinability of tumor cells usually correlate with enhanced mobility of surface lectin receptors. These changes have been monitored with fluorescence or electron microscopy.[16,22,23] The literature abounds with proposals that this greater receptor mobility in tumor cells might involve an increase in the "fluidity" of the membrane lipids. Experimental evidence to support this view has yet to be obtained.[22-24] Perhaps more important are the recent observations made in several laboratories that the content or organization of membrane-associated cytoskeletal components such as microfilaments and microtubules are usually reduced in transformed cells compared to their untransformed counterparts (reviewed elsewhere[23]). This finding might explain the loss of receptor capping on cells from chronic lymphatic leukemia patients and in other neoplastic disorders.[23]

Similarly, antigens on transformed cells are in many cases more mobile than similar antigens on untransformed cells.[23,36] These results may be due to constant sublethal proteolytic action on tumor cells, because the addition of proteolytic enzymes to untransformed cells also enhances agglutination and receptor mobility.[16,23,24] Changes in cell-surface receptor mobility due to alterations in trans-membrane linkage to cytoskeletal components is also likely, because local anesthetics or colchicine, in combination with cytochalasin B, can convert untransformed cell-surface receptors to a relatively more mobile state resembling the situation in transformed cells.[17,18]

Other Alterations at the Cell Surface

A variety of other-cell surface changes seem to be associated with transformation (Fig. 4).[21,23,24,37-39] Cancer cells are characterized by post-transformation appearance of tumor-associated antigens.[37,38] These antigens can be fetal or embryonic, viral, or antigens entirely unique to the transformed state, although many of them are present in normal tissues in low concentrations. Tumors also release or shed antigens and other glycoproteins into the surrounding media (see below). These released components can serve as blocking factors that interfere with host immunologic responses to neoplasia, or they can elicit weak humoral antibody responses that may in fact stimulate tumor growth (tumor "enhancement").[40]

Tumor cells have lowered requirements for many serum factors essential for cell growth.[32] Serum is a complex mixture of vitamins, proteases, hormones and other proteins, minerals and nutrients. A lowered cellular serum requirement for growth and maintenance can allow cell growth under conditions when normal cells would be quiescent.

Cell-Surface Changes and the Biologic Properties of Cancer Cells

We now have little insight into which of the many surface alterations of neoplastic cells described in the preceding section are functionally correlated with the in vivo capacity of these cells to grow unchecked, to invade and to metastasize and which alterations are only secondarily related to these complex biologic processes. Some of the more important biologic properties of cancer cells may well depend on the dynamics of surface receptors and their cytoplasmic control,[2,23] and the remainder of this article is devoted to a brief discussion of this possibility.

Escape from Immune Destruction

The fact that cancer cells express novel tumor-associated antigens[37,38,40] should result in their detection and destruction by the host immune apparatus. Theoretically, there are several mechanisms by which tumor cells might overcome the immune surveillance mechanisms of the host.[40,41] Of obvious relevance to the present discussion are examples in which antigenic determinants are shed from the cell surface or are "masked" or redistributed in such a way as to reduce the ability of the hosts' immune systems either to detect or to destroy (or both) the cell.

Antigenic modulation is probably the best known example in which neoplastic cells develop (or, more probably, pre-existing cells possessing those characteristics are selected for survival in the face of an immune challenge[41]) "immunoevasive" surface properties. Antigenic modulation occurs when certain tumor cells are exposed to antibodies against cell-surface antigens; they then rapidly become insensitive to the cytotoxic effects of complement,[42] which is known to require the correct display of cell-surface-bound antibody molecules.[43] Stackpole et al.[43] have examined

the dynamics of surface antigens such as thymus-leukemia (TL) antigens on mouse lymphoma cells and have found that such antigens that undergo modulation possess relatively rapid lateral mobility in the membrane and are easily redistributed after antibody binding to form patches and caps. Some endocytosis and shedding of TL-anti-TL complexes also occurs during modulation, but the majority of TL antigens remain at the cell surface under conditions of modulation. The ease with which TL antigens are redistributed might well account for tumor-cell escape from complement-mediated immunologic killing, because a correct distribution and disposition of adjacent antibody molecules bound at the cell surface is necessary to initiate fixation of complement components. Capping or patching on the one hand probably prevents complement binding by aggregating antigens and antibodies into large, immobilized complexes so that steric hindrance or antibody molecular distortions are deterred. Alternatively, lack of antigen-antibody lateral mobility would inhibit close approach of surface-bound antibody molecules, again prohibiting complement attachment. Several means of tumor-cell escape from complement-mediated immune lysis that could result from dynamic changes in ligand-receptor redistribution are shown in Figure 6. Cancer cells probably use other mechanisms (reviewed elsewhere[40,41,44]) as well to escape destruction. Cellular trans-membrane control over the dynamics of surface antigens, perhaps mediated via the cytoskeletal systems described above, may be critically important to the neoplastic-cell survival, and the altered display of surface receptors on tumor cells may aid in their recognition by the immune system or abet their escape from immune surveillance.

Another important means of tumor-cell escape from immune destruction is by shedding of antigens or antigen-antibody complexes from the cell surface (Fig. 6). These shed components or complexes make up a family of so-called "blocking factors" that interfere with host immunity against neoplasia, and their presence in the host above critical levels can result in effective neutralization of the cell-mediated arm of immunity against tumor cells.[40,44]

Alterations in Cell Adhesion; Recognition and Growth Control

Several other biologic properties of tumor cells may be influenced by the dynamics of surface receptors and their cytoplasmic control. The property of contact inhibition of cell movement that is lost after neoplastic transformation could be an example of a failure in trans-membrane communication. The locomotion of cells is controlled by their cytoskeletal assemblages, and alterations in cytoskeletal organization mediated through cell-surface trans-membrane receptor linkages to these assemblages could provide an important mechanism for the control of normal cell movement and maintenance of cell position. In this case neoplastic transformation might result in alteration of the surface receptors or patterns of receptors responsible for contact inhibition, perturbation of cytoskeletal organization or its trans-membrane communication to surface receptors, or combinations of the above.

The loss of substrate dependency (anchorage dependence) or necessary attachment of cells to a suitable surface to trigger initiation of normal cell proliferation seems to be a reliable in vitro criterion for neoplastic transformation and tumorigenicity.[45] The attachment of normal cells via their surface receptors to a suitable substrate may require redistribution of cell-surface receptors into nonrandom arrays or distributions. Communication of this surface rearrangement could occur via trans-membrane linkages to cytoskeletal components, and alterations in this communication after transformation might be involved in the release of tumor cells from substrate-dependent growth properties.

The stimulation of cell growth by serum factors, the most important being serum hormones,[32] provides yet another possible example whereby modification in the dynamics of cell-surface receptors may be implicated in the initiation of trans-membrane signals controlling essential biologic processes. As with the proposal of Cuatrecasas,[46] hormone binding to its surface receptors may not result in direct activation of intracellular enzymes such as nucleotide cyclases; further interactions between other surface receptors and inner-membrane surface components may be necessary for a cellular hormonal response. In this scheme the dynamics of surface receptors on tumor cells and the ease with which they can be redistributed into larger ligand-receptor complexes may allow neoplastic cells to be triggered to proliferate by levels of hormones and other mitogens that would fail to stimulate growth of normal cells.

Finally, the existence of specific topographic "patterns" of surface macromolecules on different cell types or on cells at different growth or developmental stages could serve as a potential mechanism for cell-to-cell recognition and adhesion.[1,24,47] Disruption or alteration of the normal topographic displays of surface components on tumor cells might render them increasingly unresponsive or refractory to the normal cellular "signals" responsible for maintaining cell positioning within tissue. The progressive loss or complete absence of such positional controls in tumor cells would enable them to invade surrounding tissues and facilitate the establishment of metastatic foci. Alterations in cell recognition and adhesion appear to have important roles in the arrest of circulating cancer cells at specific secondary sites.[48-50]

How Are Cell-Surface Topography and Trans-membrane Control Modified in Tumor Cells?

In pursuing the theme that changes in the mobility and distribution of plasma membrane components make a major contribution to the malignant phenotype,[2,23] we have still to identify the basic mechanism (or mechanisms) underlying the aberrant surface dynamics expressed by tumor cells. One possibility that

has attracted considerable attention recently concerns the question of modification of the tumor cell surface by proteolytic enzymes. In the type of membrane organization described in the first part of this article, protease-induced alteration or loss of components on the outer face of the plasma membrane could well produce conformational changes in the integral membrane proteins or glycoproteins carrying the protease-susceptible moieties, and such changes, in turn, could alter their trans-membrane "linkage" with cytoskeletal elements on the inner side of the membrane. Dislocation of integral membrane proteins and glycoproteins from cytoskeletal trans-membrane control elements would be expected to result in altered mobility and redistribution of these components. The fact that increased receptor mobility and several of the altered surface properties displayed by tumor cells can be detected in normal cells after exposure to proteases for just a few minutes,[22-24,28,31] strongly suggests that expression of these particular surface modifications in tumor cells need not involve acquisition of novel cell-surface determinants and may merely result from topographic rearrangement of already existing normal cell-surface components[2] or loss of trans-membrane control over induced rearrangements. The disruption of pre-existing linkages or topographic relations of components on the cell surface by proteases could occur as a reversible phenomenon, whose duration would be restricted by the period of active proteolysis on the cell surface. Such a mecha-

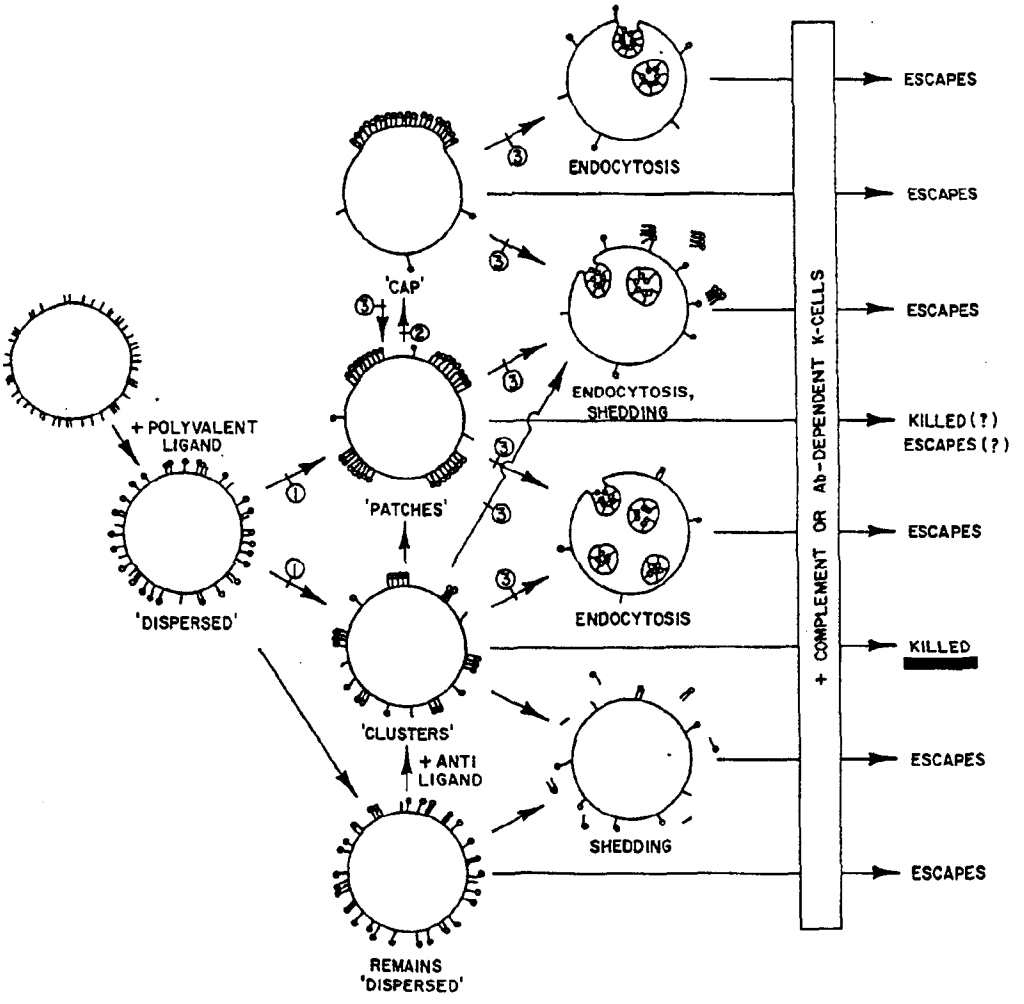

Figure 6. Some Possible Mechanisms for Tumor-Cell Escape from Immune Surveillance That Depend on Cell-Surface-Antigen Dynamics (Reproduced from Nicolson[23] with the Permission of the Publisher).

Block indicated by ① represents low temperature or chemical fixation; ② local anesthetics or colchicine combined with cytochalasin B; and ③ metabolic inhibitors. The normal pathway of antibody-complement cytotoxicity is indicated by bold underlining.

nism might explain the expression of "transformed" cell properties on normal cells during mitosis, and the continued expression of some "mitotic" surface properties in tumor cells during interphase[23] could be interpreted as indicating that proteolysis is occurring continuously. In either situation, proteolysis could modify cell-surface control mechanisms, leading to a pleiotropic stimulus that initiates a wide range of cell-surface and metabolic alterations.

Additional information is clearly required to answer several important questions. If proteolysis of the cell periphery is responsible for some aspects of the common surface characteristics and dynamics displayed both by tumor cells and by normal cells (during mitosis), we might profitably investigate the cellular mechanisms involved in regulating the release of proteases, particularly in normal cells. It also remains to be shown precisely how proteolysis of components on the external face of the plasma membrane might alter the trans-membrane linkage of membrane components to regulatory elements on the inside of the membrane. Finally, more information is needed on the range of surface alterations that can result from dislocation of linkages between integral membrane components and membrane-associated cytoskeletal elements.

In this brief outline we have attempted to summarize some of the current research on the dynamics of cell-surface organization and indicate how perturbation of the control mechanisms responsible for regulating the mobility and distribution of surface components might be of consequence in determining some of the properties of tumor cells. One potentially important implication that emerges from this work is that expression of certain surface alterations need not involve acquisition of uniquely "tumor-specific" surface components but could equally well arise simply from topographic rearrangement of existing surface components also present on normal cells. Also implicit in this proposal is the concept that the topographic "patterns" of cell-surface molecules found on different cells could act as a "code" by which normal cells recognize each other and regulate their growth and social behavior and that alterations in such topographic patterns, if sustained, could result in the spectrum of aberrant behavioral characteristics displayed by cancer cells. Although a high degree of speculation must currently surround the interpretation of information on this subject, sufficient experimental data are already available to support a functional relation between the topography and dynamics of surface macromolecules and the control of cell-surface properties. It is therefore anticipated that future research on the mechanisms regulating the dynamics of cell-surface organization will yield important insights into how the cell surface influences the growth and social behavior of cells, and it is expected this information will be of relevance to both morphogenesis and cancer.

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