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This report is: ☐ INTERIM ☐ FINAL and mainly: ☐ NEW ☐ REVIEW

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DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

A general review of chemical carcinogenesis with perspective on the impact of chemobiokinetics is presented. Examples are cited which suggest that the interaction of chemical carcinogens with DNA and subsequent repair processes are dose-dependent. Thus, at high dose or exposure levels the carcinogenicity of many chemicals is enhanced because the normal detoxification mechanisms and the normal DNA repair mechanisms are overwhelmed. Therefore, while not invoking a threshold concept, it becomes clear why the dose-response function deviates from a linear relationship at low doses. Extrapolation of carcinogenicity from exaggerated high doses where normal detoxification and repair processes are impaired to low doses leads to an unrealistic over-estimate of risk to low level exposures. Only by understanding the relationship between chemobiokinetics and carcinogenesis will it be possible to make meaningful evaluations of the hazard of exposure to carcinogens for man.

Presented at the "International Symposium on Industrial Toxicology" University of Surrey August 25-30, 1977.

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CHEMOBIOKINETIC PERSPECTIVES ON MECHANISMS OF CHEMICAL CARCINOGENESIS

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Mechanisms of carcinogenesis have been studied xtensively in the past. Ionizing radiation has been the primary model for studying these mechanisms and from this model arose the concept of linear extrapolation of effect with the exposure dose. However, even for ionizing radiation evidence exists showing that there is a no effect exposure level for people exposed to radium (Evans, 1974).

Chemical carcinogenesis differs significantly from ionizing radiation in that chemicals are absorbed, distributed and biotransformed (activated and detoxified) in the body and excreted as the chemical per se or as biotransformation products. Collectively, the study of the time related sequence of the overall fate of a chemical in the body is termed chemobiokinetics. The importance of considering chemobiokinetic concepts in evaluating the toxicological hazard of exposure to chemicals has been reviewed recently (Gehring, 1978, this volume). More specifically, chemobiokinetics has given insight into why it is inappropriate in certain circumstances to predict the same toxic effects observed at high doses to much lower doses. Linear extrapolation from high to low doses is a highly controversial issue in assessing carcinogenic hazard. The ensuing discussion on mechanisms of carcinogenesis will reflect this chemobiokinetic perspective and deal with the relationship between the reaction of chemicals with intracellular macromolecules and correlation with toxicity and carcinogenicity. Examples will be given where alteration of the chemobiokinetics of a chemical or its alkylated products may cause the

dose-response curve to deviate from linearity at v ry high and/or very low dose levels. These deviations from linear dose-response relationship are important both in study design and in the interpretation of toxicity data used for assessing potential risk of chemical exposure.

Background on Chemical Carcinogenesis

The somatic mutation theory is probably the most attractive working hypothesis in the field of chemical carcinogenesis. Evidence in support of this hypothesis includes the following observations: 1) many chemical carcinogens induce mutations; 2) the long latent period between chemical exposure and the manifestation of cancer is consistent with effects in a macromolecule such as DNA which is capable of retaining information over a long period of time; 3) genetic diseases with associated chromosomal abnormalities are often associated with an increased incidence of cancer and 4) many chemical carcinogens interact with DNA.

The somatic mutation theory of carcinogenesis was advanced considerably by the work of the Miller's (1966, 1970) when they demonstrated that many chemical carcinogens are either electrophiles (direct acting) or metabolized in vivo to an electrophilic species (indirect acting). Figure 1 shows several examples of direct and indirect acting alkylating agents. The alkylating agents are a large, chemically diverse group of chemicals. β-Propiolactone is a direct acting alkylating agent which will react with nucleophiles. In contrast dimethylnitrosamine and 2-acetylaminofluorene are examples of chemicals which require metabolic biotransformation to their

electrophilic forms before any reaction with a nucleophilic site can occur. The two generally recognized mechanisms of alkylation are first and second order nucleophilic substitutions denoted SN_1 and SN_2 respectively. β-Propiolactone and 2-acetylaminofluorene presumably undergo second order nucleophilic substitution (SN_2) reactions while formation of the methyl carbonium ion from dimethyl nitrosamine would be described by a unimolecular nucleophilic reaction (SN_1). The positive, electrophilic atoms of the ultimate carcinogen react with the relatively negative or nucleophilic atoms of the molecule being attacked.

Targets for attack of electrophilic reactants on the DNA molecule include the four bases and in some instances the phosphodiester backbone. Nucleophilic centers of DNA bases which have been reported to be alkylated are O-6, N-3, 2-NH_2, and C-8 of guanine; 6-NH_2, N-1, N-3 and N-7 of adenine; N-3 and C-5 of cytosine; and N-3, O-4 and C-6 of thymine Figure 2 (Sarma et al., 1975). The most reactive groups are the purine nitrogens. The N-7 position of guanine is the most reactive site followed by the N-3 and N-7 positions of adenine. The high reactivity of the N-7 position of guanine has been attributed to its accessibility by its position in the wide groove of the Watson-Crick Model and its favorable high electron density over the purine ring (Fishbein, et al., 1970). The nucleophilic sites of the purine bases and their hydrogen bonding relationships in double stranded DNA are shown in Figure 3. The N-7 position of guanine, the most nucleophilic site,

is not involved in hydrogen bonding, but it has been shown that alkylation of this position can cause depurination of the DNA which can lead to death (Fishbein, et al., 1970). Attempts to correlate the carcinogenicity of alkylating agents with the extent of the reaction at the N-7 position have not been successful (Swann and Magee, 1968; 1971). However, the persistence of the alkylated product at the O-6 position of guanine, a site involved in hydrogen bonding, has been correlated with the organ specific carcinogenicity of ethylnitrosourea and dimethylnitrosamine (Goth and Rajewski, 1974 a,b; Pegg et al., 1976). This work has generated considerable interest in O-6 alkylation of guanine as a plausible mechanism of carcinogenesis.

An important characteristic of chemical carcinogenesis is that it is a multistage process. A chemical must be absorbed, activated (if necessary), distributed to target sites, react with macromolecules at critical sites and cause cellular transformation to a neooplastic cell. Finally the transformed cells must proliferate to produce a malignant neoplasm. Each of these steps leading to cancer has one or more competing processes which would inhibit the progression towards a neoplastic event. Upon absorption the chemical or its reactive species can be excreted or detoxified or react with non-critical receptors such as structural proteins or non-critical sites on nucleic acids which lead to no deleterious effect. Furthermore, reactions leading to cell death would also inhibit the progression towards cancer.

Once a chemical has penetrated the cells of the target organ and reacted with critical sites on DNA, numerous repair mechanisms can repair the damage. Mammalian cells have been shown to effectively undergo excision repair, post-replication repair and repair of single and double strand breaks (Trosko and Chu, 1975). Different tissues have different capabilities to repair DNA and this implies potential organ specificity for induction of tumors. In addition, once a cell is transformed there is evidence that an immunologic surveillance mechanism exists which destroys abnormal cells through antigenic stimulation of the cellular immune (T-cell) response (Baldwin, 1973; Melief and Schwartz, 1975; Old 1977).

If all of the steps leading to cancer and all of the competing reactions tending to nullify the carcinogenic response were governed by first order kinetics over a given dose range (i.e. the reaction rates were a direct function of the concentration of the reactants), then the dose-response curve for induction of tumors would be linear throughout the dose range. Subsequently, if the population studied were distributed normally, a sigmoid cumulative dose-response curve would result (Figure 4). However, evidence suggests that toxicity, including carcinogenicity, may be manifested in many instances only after excretion, repair or other natural defense mechanisms are saturated at high dosage levels (Gehring, et al., 1976; 1978). In such instances, the dose-response curve will deviate from its linear relationship and the risk of incurring cancer at low doses may be much less than predicted from

extrapolation of the incidence of cancer incurred by administration of high doses. This is indicated by the various extrapolated lines (dashed lines) in the hypothetical dose-response curve below levels where carcinogenic responses can be measured accurately (Figure 4). In practice, toxic effects are determined following high doses of a chemical. While this approach is valid for determining the maximum response, use of these data to assess responses at much lower dose levels is limited severely without adequate chemobiokinetic and/or biochemical evidence indicating that the kinetics of the reaction leading to toxicity do not change dramatically with increasing dose. Examples illustrating a correlation between the reaction of chemicals with DNA at high doses and resultant carcinogenicity follow.

Persistence of O^6-alkylated Guanine and Carcinogenesis

Goth and Rejewsky (1974, a,b) demonstrated the correlation between organ specific carcinogenicity and the alkylation of the O-6 position of guanine. N-ethyl-N-nitrosourea (ENU) yields an electrophilic ethyl cation via a non-enzymatic heterolytic decomposition. Single administration of ENU to newborn rats (10-20 days old) results in a high incidence of malignant tumors of the central and peripheral nervous system. DNA replication and cell division appear to be related to the carcinogenic response since maximum sensitivity to ENU (perinatal age) is characterized by a highly proliferative state of rat nervous tissue.

Following intraperitoneal administration of 75 mg/kg ENU in perinatal rats, various alkylated bases were isolated from liver and brain. The liver is not sensitive to the carcinogenic action of ENU under these conditions. The clearance of N-7 ethylguanine from liver and brain expressed as its ratio with guanine is shown over a 240 hour period (Fig. 5). The interest in the N-7 position of guanine is due to the fact that this is the most nucleophilic site of the four DNA bases and thus reaction at this position is greatest. The half-life for N-7 ethylguanine in liver and brain were 64 and 89 hours respectively. In contrast, the clearance of O-6 ethylguanine from liver and brain gave half-lives of 36 and 229 hours respectively (Figure 6). Thus it appears that the sensitivity of the nervous tissue is associated with the persistence of the O-6 ethylated product of ENU and guanine.

Of the various sites of potential alkylation of DNA bases the O-6 position of guanine is a particularly attractive candidate for causing mistakes in base pairing because alkylation of the oxygen forces guanine into its enol form (Lawley and Thatcher 1970; Lawley and Shah, 1972; Kleihues and Magee, 1973). This may cause O-6 ethylguanine to pair with thymine rather than cytosine. It has been shown by Gerchman and Ludlum (1973) that O-6 alkylation results in misincorporation of bases in a bacterial nucleic acid polymerase system. In contrast N-7 methylguanine showed normal G-C pairing in a similar system. In the case of ENU the N-7 ethylguanine may cause lethal cellular effects due to the resulting depurination of DNA. This would result in cellular death which would terminate the process leading to

neoplasm. From these data it appears that the persistence of O-6 ethylguanine due to the reduced capacity for repair in the nervous tissue resulting from a poor capability to excise O-6-ethylguanine from DNA correlates well with tumor specificity for the nervous system.

The next example illustrates the dose-dependent clearance of O-6 methyl guanine from the kidney of rats treated with dimethylnitrosamine (DMN) and an associated organ specific carcinogenic effect. This work was conducted by Pegg, et al. (1976, 1977) and is an extension of previous work by Magee (1962) Magee and Farber (1962) Swann and Magee (1968) and Lawley et al. (1968). Administration of a large single dose of DMN induces kidney tumors in rats; single administration of DMN does not induce liver cancer. Following a single intraperitoneal dose of 2.5 or 20 mg/kg DMN alkylated bases of DNA were isolated from the liver and kidney. The clearance of N-7 methylguanine, expressed as its ratio to guanine, over a 120 hour period was similar in both liver and kidney (Figure 7). Furthermore, the N-7-methylguanine content in both liver and kidney appeared to increase in direct proportion with the increase in dose.

In contrast to this profile, the clearance of O-6 methylguanine, expressed as its ratio to guanine, was markedly dose-dependent in the kidney (Figure 8). Following the 20 mg/kg dose the ability to eliminate the O-6 methylguanine, presumably by

DNA excision repair mechanisms in the kidney, is markedly inhibited. Persistence of the O-6 methylguanine correlates with the induction of kidney tumors suggesting that the inability to repair the O-6 alkylated guanine following a high dose of DMN may result in induction of cancer. Furthermore, any extrapolation to predict the incidence of kidney tumors at lower doses where DNA repair is not compromised would overestimate the risk. The saturation of the DNA repair mechanism leading to a deviation from linear kinetics would thus cause a deviation in the dose-response curve at low level exposure.

It appears in the foregoing examples for DMN and ENU that the induction of tumors in rats correlates reasonably well with persistence of O-6 alkylated guanine. However, like many initial hypotheses to describe a complex biological process, additional evidence now suggests that other factors may also play a role in the carcinogenic response to DMN in addition to the persistent O-6 alkylated product of guanine. Recent studies on the induction of liver tumors in rats following repeated oral administration of DMN do not correlate as well with the reaction and persistence of O-6 alkylated guanine as do kidney tumors following intraperitoneal

injection (Buecheler and Kleihues, 1977; Nicoll et al., 1977). Nonetheless, the intraperitoneal studies have given insight into one mechanism which is consistent with the induction of tumors in a sensitive tissue due to the saturation of DNA repair. The high susceptibility of xeroderma pigmentosium patients to UV light-induced squamous cell carcinoma due to their inherent lack of DNA excision repair strongly supports the concept that interference with normal DNA repair mechanisms causes a predisposition to cancer. (Cleaver, 1977).

The future challenge will be to elucidate which sites in the DNA, or for that matter RNA and protein as well, are critical for initiation of the neoplastic process and be able to relate the kinetics of those reactions and subsequent repair to the dose-response to carcinogenesis. In a given case it may be the O-6,or N-7 position of guanine or any of the other nucleophilic sites which have been identified thus far.

Dose-response Considerations for Chemicals Requiring Metabolic Activation

Numerous studies have been conducted on the chemobiokinetics of vinyl chloride (Bolt et al. 1977; Green and Hathway 1975 Watanabe et al. 1976 a,b) It has been postulated that vinyl chloride (VC) is biotransformed to a reactive metabolite which reacts presumably with intracellular macromolecules resulting in carcinogenesis

(Van Duuren, 1975; Hefner, et al., 1975). Furthermore, it appears that the biotransformation of VC to this reactive metabolite is a saturable process. (Green and Hathway, 1975; Bolt, et al., 1976; Watanabe, et al. 1976, a,b). Recently in our laboratory evidence has been obtained which correlates the carcinogenesis of vinyl chloride in rats with the reaction of VC metabolites with hepatic macromolecules.

In these studies rats were exposed under dynamic conditions to atmospheres of ^{14}C-labeled VC for a 6 hour period. Following a 6-hour exposure to various concentrations of VC ranging from 1-5000 ppm the rats were killed and the macromolecular binding of radioactivity in the liver was determined by TCA precipitation and subsequent exhaustive solvent extraction. The binding results are summarized in Figure 9 (Watanabe, et al., 1978). The triangles show the decrease of hepatic GSH levels as the exposure concentration increases. Covalent binding of ^{14}C-activity to hepatic macromolecules plotted as a function of the log of the exposure concentration was best represented by a sigmoid shaped curve. The linear portion of the binding curve extended from about 50-500 ppm. At exposures exceeding 500 ppm the macromolecular binding in the liver appears to approach a plateau.

A plot of the incidence of hepatic hemangiosarcomas in rats exposed to VC at concentrations greater than 50 ppm is shown in Figure 10. The percent incidence of hepatic angiosarcoma

from data by Maltoni (1977) is shown by the open squares. The tumor incidence parallels the increase in binding of VC metabolites to intracellular macromolecules; above 500 ppm the tumor incidence as well as degree of binding appear to plateau.

The relationship between the binding of VC in the liver and the amount of VC biotransformed is shown in Figure 11. The binding of radioactivity to liver macromolecules is directly related to the amount of VC biotransformed supporting the hypothesis that reactive metabolites of VC are produced in vivo and are responsible for the induction of tumors. Thus the principles presented and discussed by Gehring (1978, this symposium) relating the metabolism of VC to carcinogenesis are also applicable to the reaction of VC with tissue macromolecules.

If Michaelis-Menten kinetics were applied to the saturable covalent binding of radioactivity to hepatic macromolecules, a plot of the log velocity of the binding reaction versus the percent induction of hepatic angiosarcomas in rats would result in a linear function. In contrast, if the tumor incidence is plotted as a function of exposure concentration the resulting relationship is not linear. The reason for the differences in the dose-response curves is that in the case of VC the biotransformation to a reactive species which binds with macromolecules is a saturable process. Thus a linear dose-response function results only when carcinogenicity is related to the formation of the reactive metabolite,

which in this case requires application of Michaelis-Menten (saturable) kinetics. Failure to consider these pharamcokinetic aspects in either study design or interpretation of data relative to assessing the risk of exposure by predicting the response in a population outside the experimentally observable range can lead to gross overestimates of risk.

Secondary Carcinogenesis. Secondary or indirect carcinogenesis is a term which has been used to refer to the induction of cancer after overt damage has occurred in the sensitive tissue. One manifestation of this phenomenon is the increased incidence of tumors in partially hepatectomized animals following administration of hepatocarcinogens (Marquardt et al., 1970; Craddock, 1971; Date et al., 1976). Additional evidence supporting the hypothesis of increased susceptibility to cancer in tissues which are undergoing cellular division is the increased mutagenesis induced in synchronized cultured liver cells which are in S phase (stage of DNA synthesis) as compared to liver cells in other quiescent stages of cellular division (Williams, 1976).

Studies in our laboratory have suggested that the tumor induction in experimental animals following exposure to vinylidene chloride (VDC) is a result of secondary carcinogenesis. Inhalation exposure to VDC produces acute hepatic and renal toxicity in experimental animals. Correlation of the chemobiokinetic properties

of VDC with toxicity has led to the hypoth sis that the hepatotoxicity of VDC is mediated by a reactive electrophilic metabolite produced in vivo (McKenna, et al., 1978). At low levels of exposure this reactive intermediate is detoxified by conjugation with glutathione (GSH). Exposure to toxic concentrations of VDC results in depletion of hepatic GSH and consequently an increased alkylation of tissue macromolecules rather than GSH. This enhanced reaction to tissue macromolecules at high doses results in toxicity.

To test this hypothesis, rats were exposed to concentrations of ^{14}C-VDC ranging from 5 to 200 ppm. Exposure duration was 6 hr. Immediately after exposure the animals were killed and hepatic GSH levels and covalent binding of ^{14}C-activity to hepatic tissue were determined.

The covalent binding of reactive VDC metabolites versus the exposure concentration is shown in Figure 12. Note that once GSH concentrations are depleted the binding increases dramatically. There is no indication that this curve will bend over at higher doses like that seen with VC. This increasing binding with VDC will continue resulting in acute hepatotoxicity. These data are consistent with the hypothesis that covalent binding of VDC metabolites to tissue macromolecules represents a biochemical event which precedes the development of VDC-hepatoxicity.

Chronic toxicity studies conducted in rats exposed by inhalation to daily concentrations of 75 ppm VDC have shown no indication of a carcinogenic response (Rampy, L. W. et al., 1977). The primary toxicity observed was an effect in the liver which was reversible. However, two independent studies reported recently by Drs. Maltoni and Lee have found VDC to be carcinogenic in mice. Maltoni (1977) has reported kidney tumors in Swiss mice exposed chronically to 25 ppm, and Lee (1976) has observed hepatomas, angiosarcoma of the liver and pulmonary adenomas in CD-1 mice exposed to 55 ppm VDC for one year. Despite the marked difference in the response of the two mouse strains employed in these studies, both investigators have reported moderate to severe chronic tissue damage indicative of VDC toxicity in the tumor-bearing organs. Only in the studies of Maltoni (1977) was a "no-effect" level for VDC-induced renal damage realized (10 ppm) and at this exposure concentration no kidney tumors were observed.

In light of the tumors observed in mice and none in rats some preliminary chemobiokinetic studies were conducted in mice to compare with the existing rat data. Table 1 shows the disposition of ^{14}C-VDC in rats and mice following exposure to 10 ppm ^{14}C-VCD. While the routes of excretion between the two species were similar when expressed on a percent excreted basis, the

striking difference noted was that the mouse metabolized much more than the rat. The single 6 hour exposure to ^{14}C-VDC resulted in a body burden of 5.3 mg eq. ^{14}C-VDC/kg in the mouse, nearly twice that obtained in the rat in an identical experiment. The higher body burden and more rapid metabolism of VDC by mice suggested that production of toxic metabolites of VDC by mice may be greater than that observed in rats and thus render them more susceptible to the effects of VDC exposure.

A comparison of values obtained for covalently bound ^{14}C-activity in liver and kidney of mice and rats immediately following ^{14}C-VDC exposure is shown (Table 2). This data indicates clearly the enhanced production of reactive metabolites of VDC in mice as evidenced by the marked increase in covalently bound ^{14}C-activity in both liver and kidney when compared to the rat. In the context of previous findings regarding the relationship between covalent binding and VDC-induced tissue damage in rats, the data indicate that the mouse is much more susceptible than the rat to the adverse effects of VDC. These findings suggest that accumulation of VDC metabolites covalently bound to target organ macromolecules may be sufficient to produce tissue damage and necrosis appreciably sooner than the onset of neoplasia. To date this hypothesis is supported by the findings reported by both Maltoni (1977) and Lee (1977). In neither of these studies were tumors in mice observed in the absence of non-tumor pathology attributed to VDC exposure.

Conclusions. The examples cited were selected to emphasize the importance of understanding the chemobiokinetic reactions which may lead the production or alteration of a carcinogenic response. As we begin to understand the relationships between chemobiokinetics, carcinogenesis and ultimately mechanisms of carcinogenesis it should enhance our ability to make meaningfull evaluations of the hazard of exposure to carcinogens in man.

LEGENDS

Figure 1. Examples of indirect and direct acting electrophilic carcinogenic chemicals.

Figure 2. Nucleophilic centers of DNA nucleic acid bases

Figure 3. Base pairing characteristics of nucleic acids of DNA.

Figure 4. Hypothetical log dose versus percent response curve. Measurable responses are represented by triangles. The sigmoid curve (---) represents a population described by a normal distribution; in theory the percent response approaches 0 and 100% asymptotically. The other curves (----) and (-•-•-•) represent simulated responses if there exists a threshold for the response.

Figure 5. Mole fraction of N-7-ethylguanine/guanine in the DNA of brain and liver as a function of time following a 75 mg/kg intraperitoneal injection of ethylnitrosourea in 10 day old rats.

Figure 6. Mole fraction of O-6-ethylguanine/guanine in the DNA of brain and liver as a function of time following a 75 mg/kg intraperitoneal injection of ethylnitrosourea in 10 day old rats.

Figure 7. Mole fraction of N-7 methylguanine/guanine in DNA of liver and kidney as a function of time following 2.5 or 20 mg/kg intraperitoneal injection of dimethylnitrosamine in rats.

Figure 8. Mole fraction of O-6-methylguanine/guanine in DNA of liver and kidney as a function of time following 2.5 or 20 mg/kg intraperitoneal injection of dimethylnitrosamine.

Figure 9. Hepatic macromolecular binding and glutahione (GSH) depression as a function of the logarithmn of the exposure concentration to vinyl chloride (VC): Macromolecular binding (●) µg equivalents VC bound per g protein (mean ± S.D.) GSH (▲) per cent of control (Watanabe et al., 1978).

Figure 10. Hepatic macromolecular binding and percent incidence of angiosarcoma (Maltoni, 1977) in rats versus the logarithmn of the exposure concentration of vinyl chloride (VC, ppm). Macromolecular binding (●) μ mole equivalents VC per g protein. Incidence of hepatic angiosarcomas (σ) percent.

Figure 11. Relationship between the amount of vinyl chloride (VC) metabolized versus the amount of VC bound to macromolecules per g liver protein (Watanabe et al., 1978).

Figure 12. Hepatic macromolecular binding and non-protein sulfhydryl (NPSH, primarily glutathione) depression as a function of the logarithmn of the exposure concentration to vinylidene chloride (VDC) in rats. Macromolecular binding (o) μ mole VDC bound per g liver protein. Non-protein sulfhydryl (NPSH, primarily glutathione) (●) percent of control (McKenna et al., 1978).

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TABLE 1

DISPOSITION OF ^{14}C-ACTIVITY IN RATS AND MICE FOLLOWING INHALATION EXPOSURE TO 10 PPM ^{14}C-VDC

	% Body Burden ($\bar{X}$ + S.E., n=4)	
	Mice	Rats
Expired VDC	0.65 ± 0.07	1.63 ± 0.14
$^{14}CO_2$	4.64 ± 0.17	8.74 ± 3.72
Urine	80.83 ± 1.68	74.72 ± 2.30
Feces	6.58 ± 0.81	9.73 ± 0.10
Carcass	5.46 ± 0.41	4.75 ± 0.78
Cage Wash	1.83 ± 0.84	0.44 ± 0.28
	(mg Eq ^{14}C-VDC/Kg)	
Body Burden	5.30 ± 0.75	2.89 ± 0.24
Total Metabolized VDC	5.27 ± 0.74	2.84 ± 0.26

McKenna et al. (1978)

TABLE 2

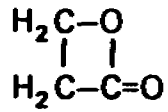
COVALENTLY BOUND ^{14}C-ACTIVITY IN RAT AND MOUSE TISSUE FOLLOWING EXPOSURE TO 10 PPM ^{14}C-VDC

	µg Eq ^{14}C-VDC/gm Protein ($\bar{X}$+S.E., n=4)	
	Liver	Kidney
Mice	22.29 ± 3.77	79.55 ± 19.11
Rats	5.28 ± 0.14	13.14 ± 1.25

McKenna et al. (1978)

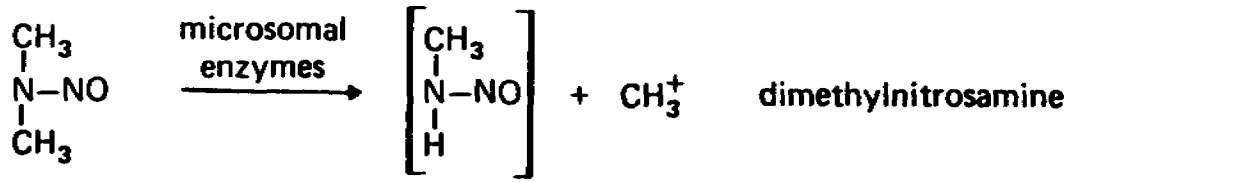
Figure 1

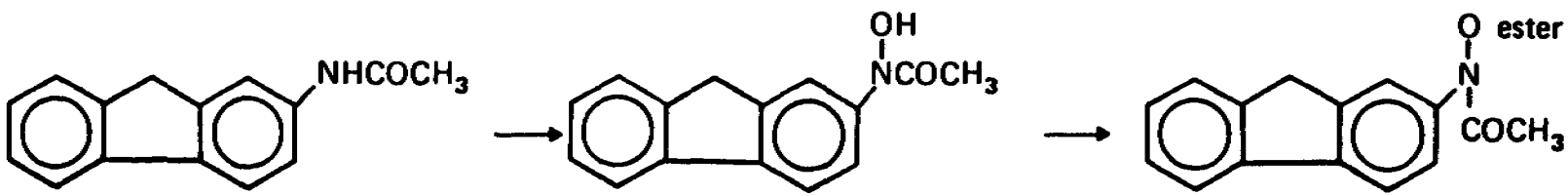
Direct Acting:



beta-propiolactone

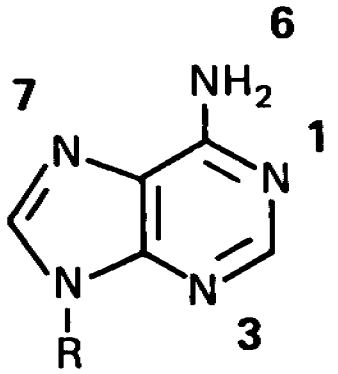
Indirect Acting:



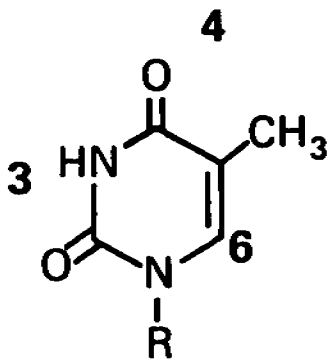


2-acetylaminofluorene

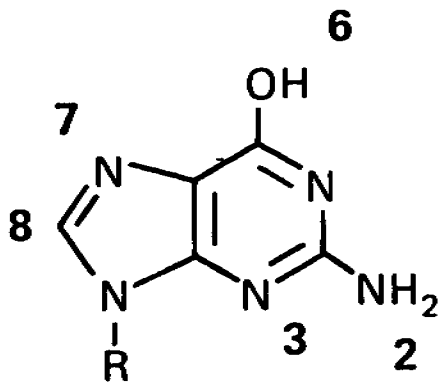
Figure 2



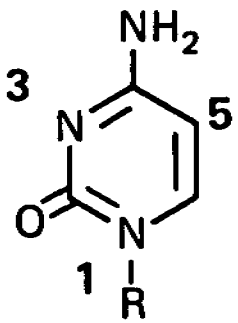
Adenine



Thymine

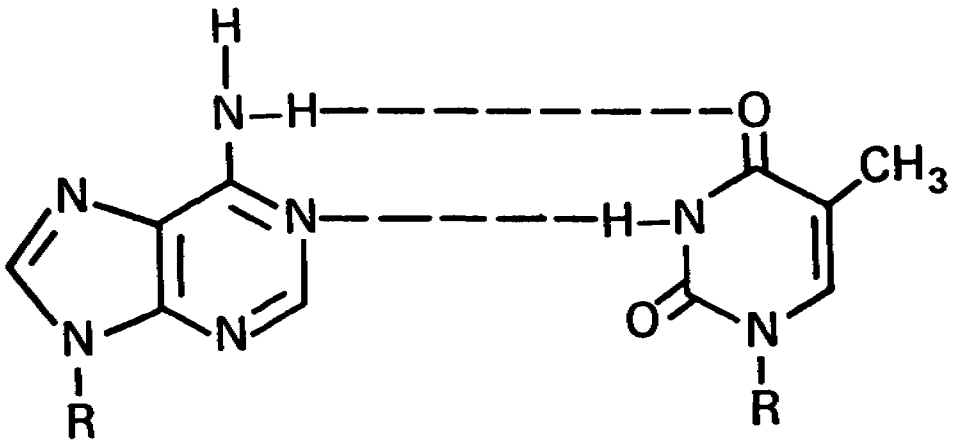


Guanine

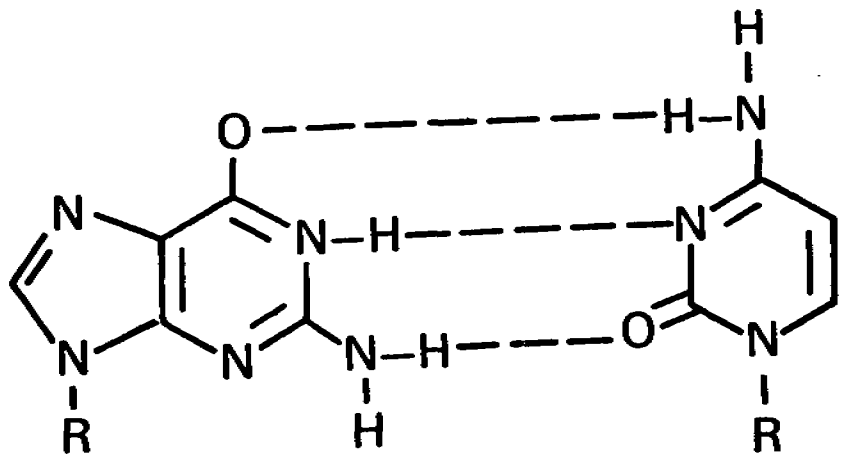


Cytosine

Figure 3



Adenine-thymine



Guanine-cytosine

Figure 4

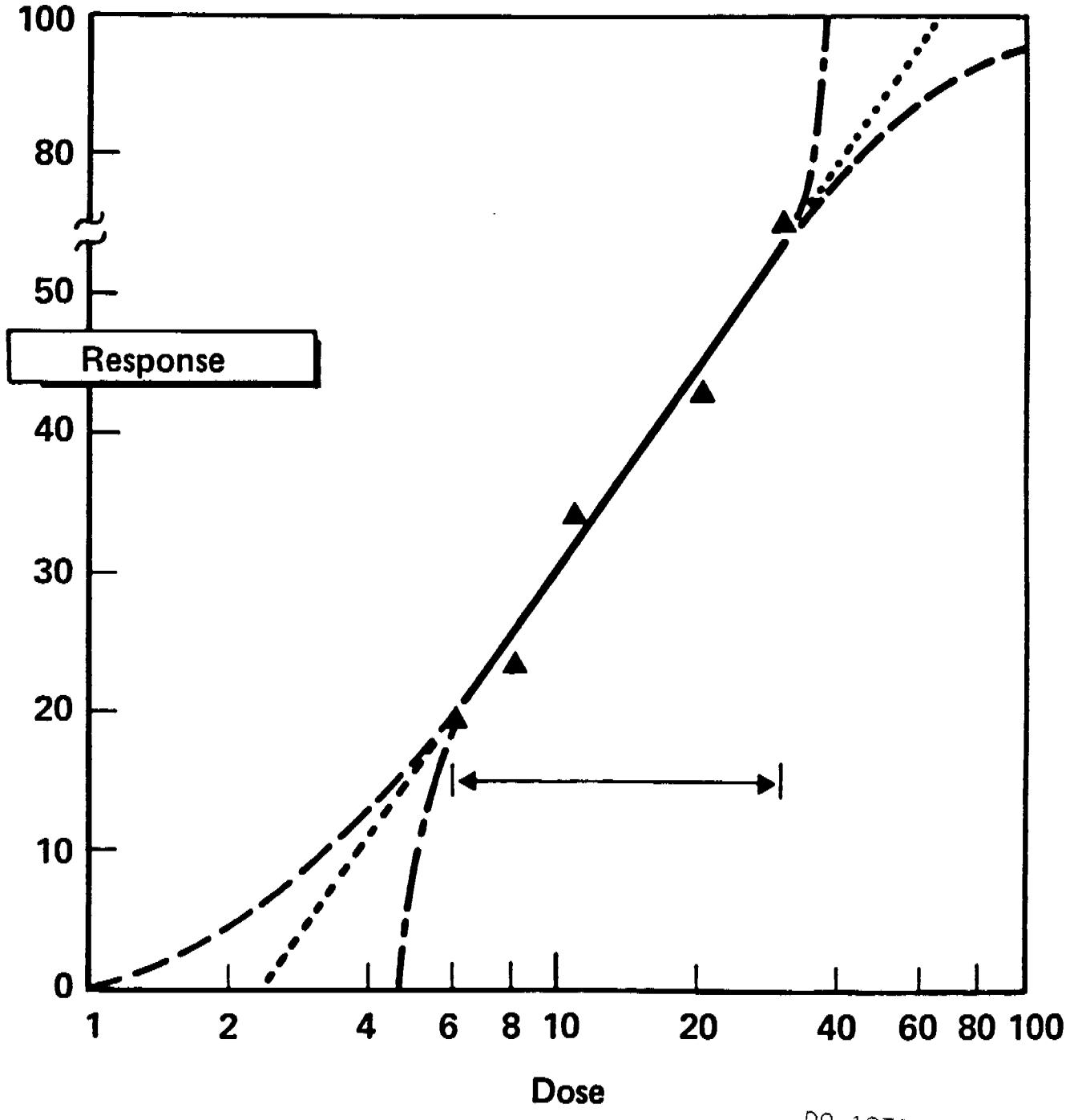
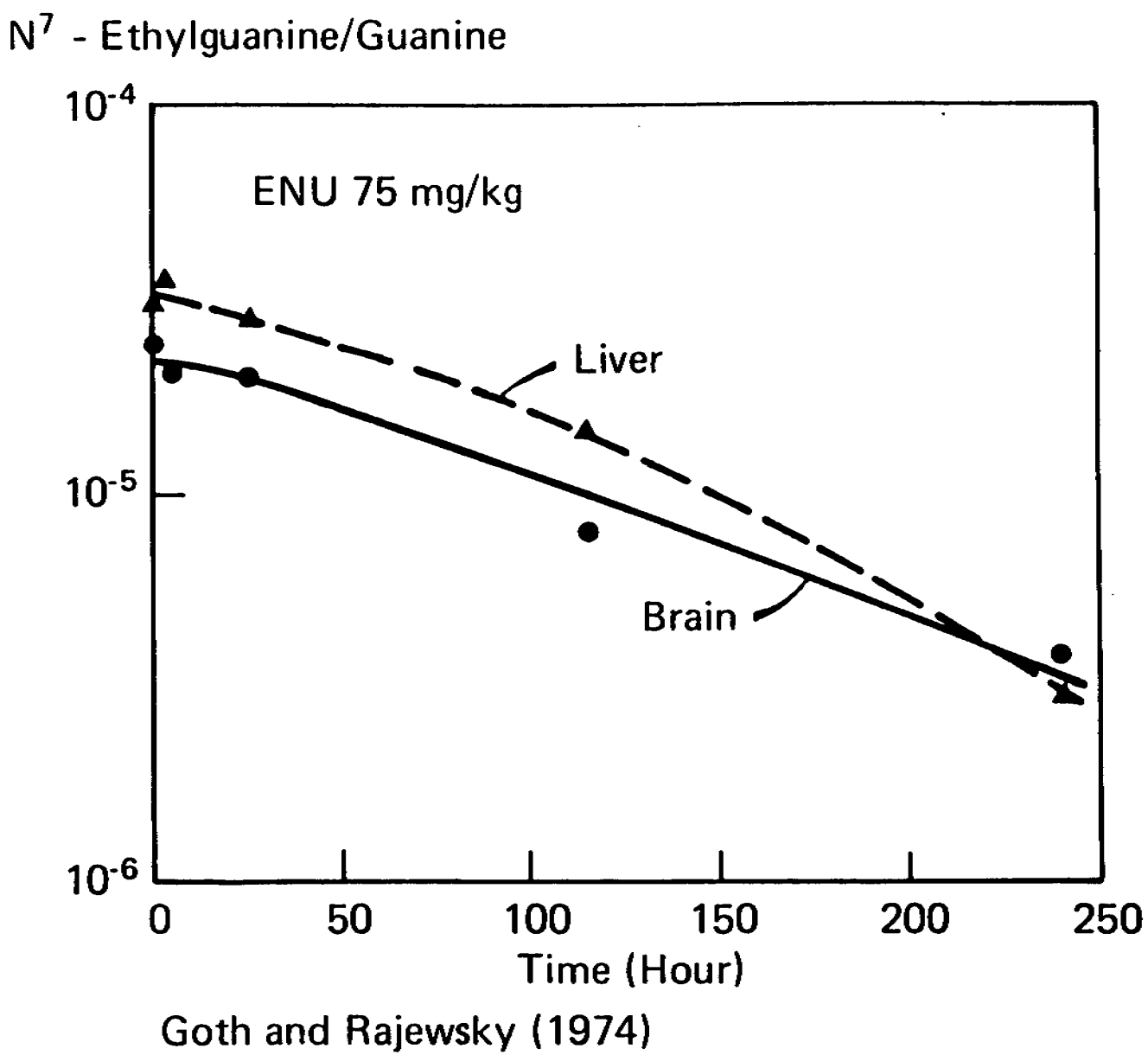
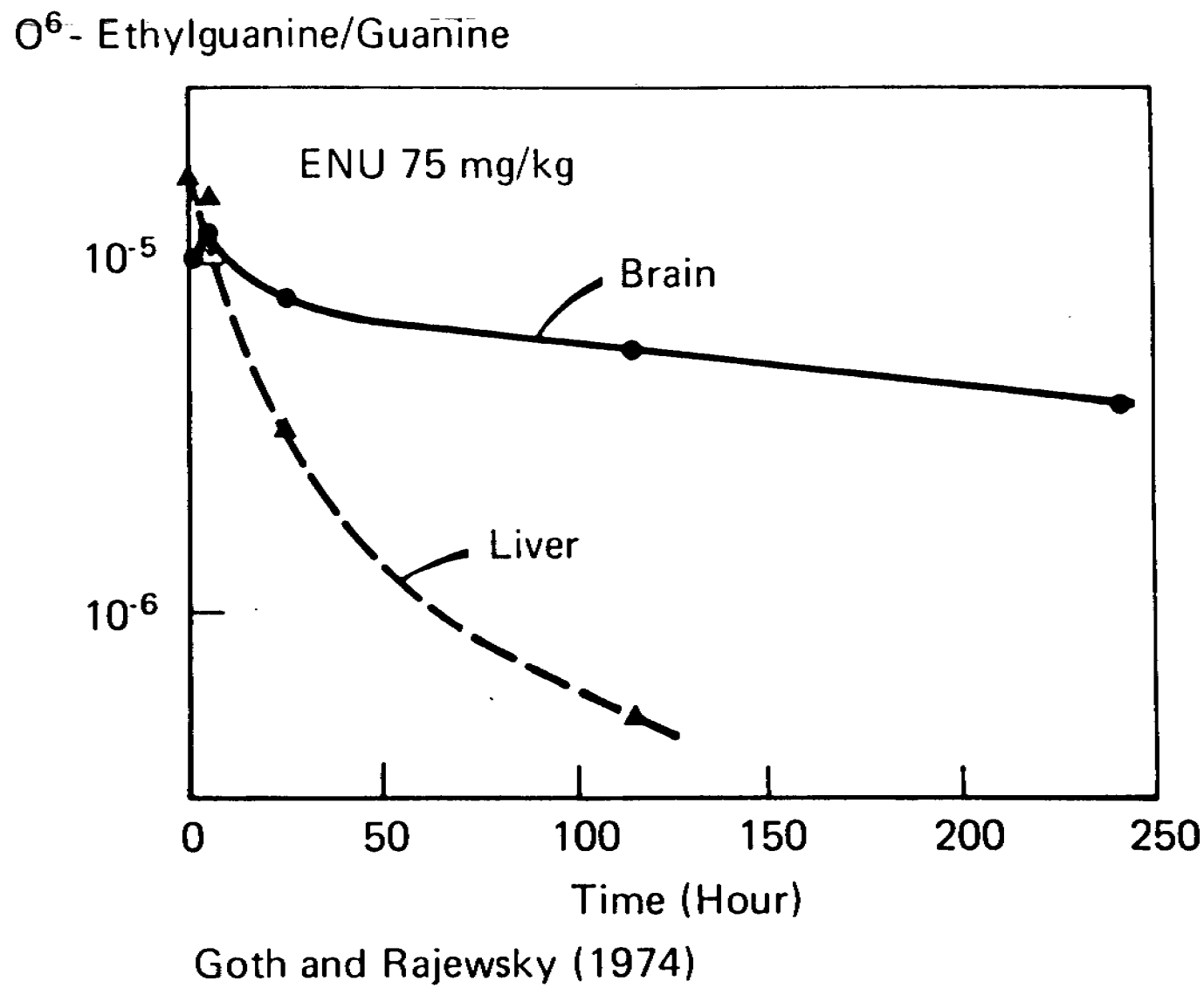


Figure 5



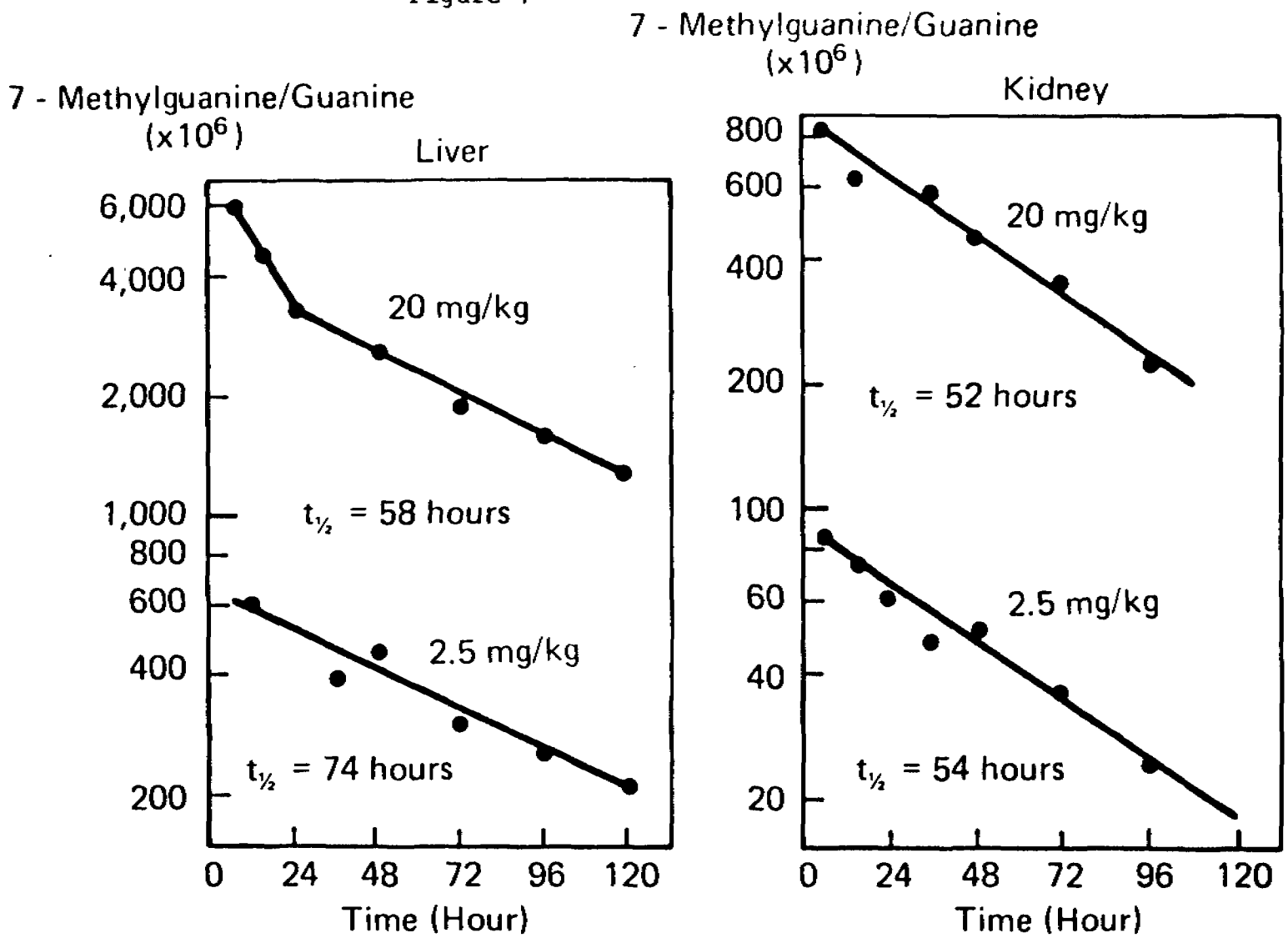
Goth and Rajewsky (1974)

Figure 6



Goth and Rajewsky (1974)

Figure 7



Pegg et al (1976)

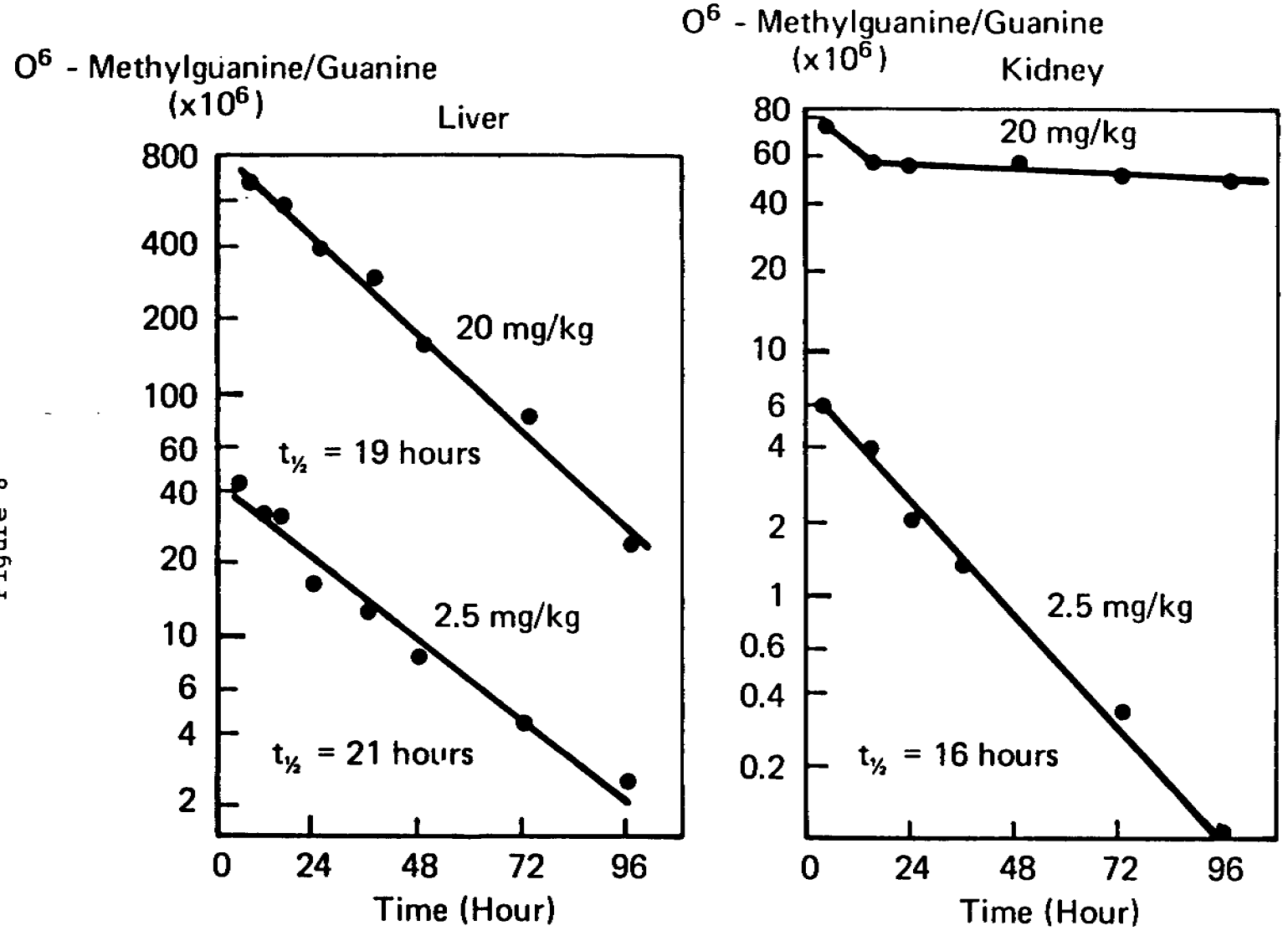


Figure 8

Pegg et al (1976)

Figure 9

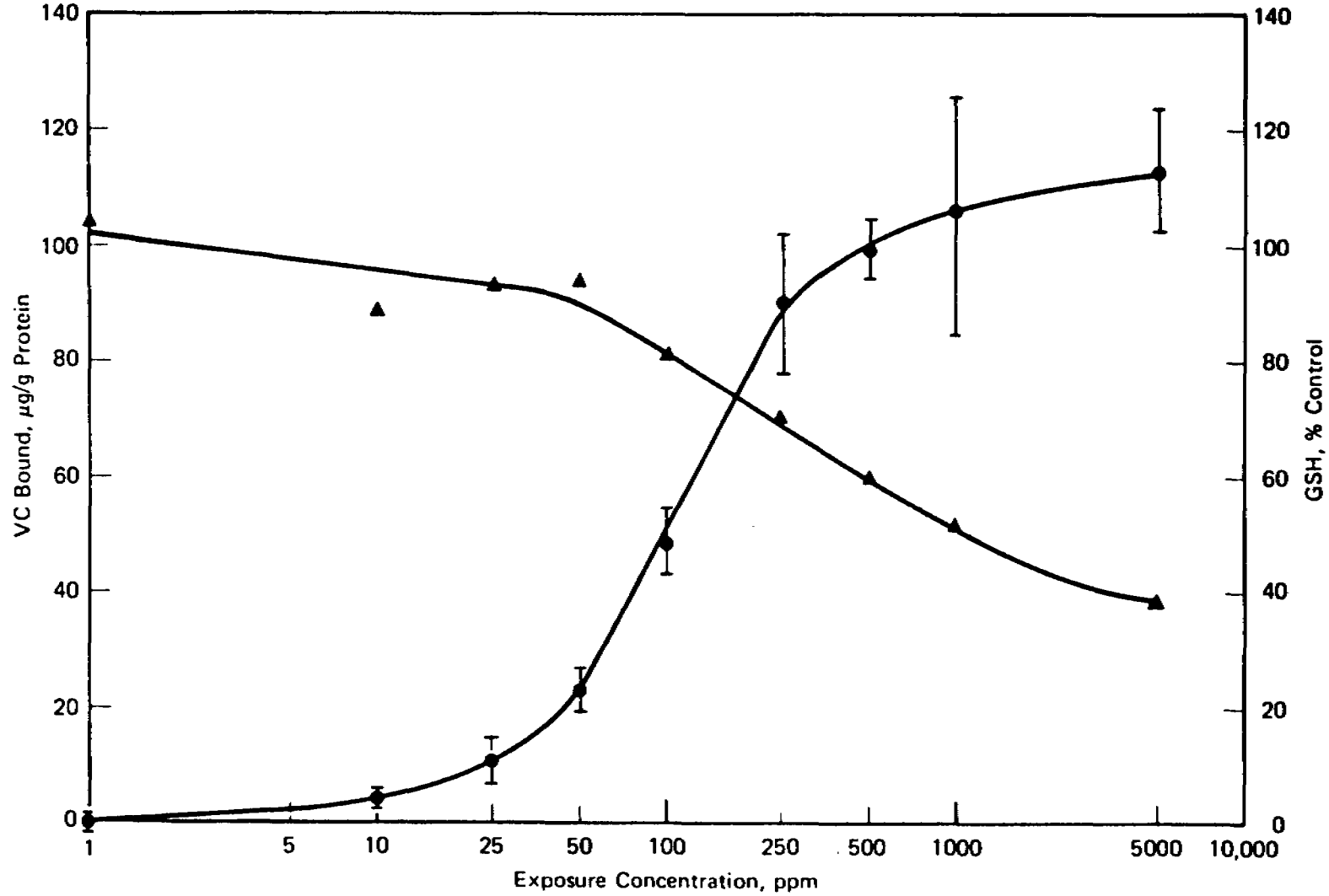


Figure 10

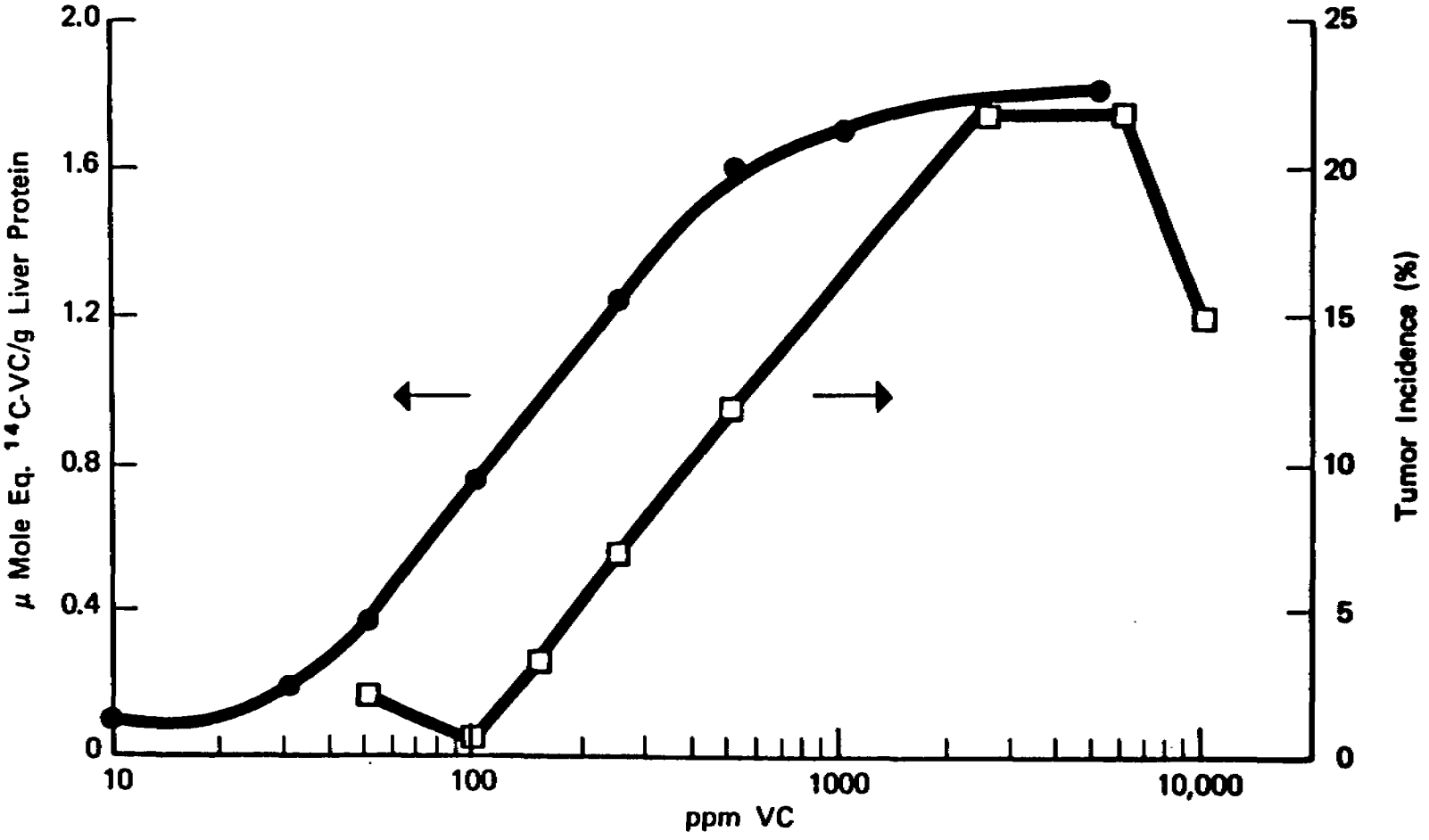


Figure 11

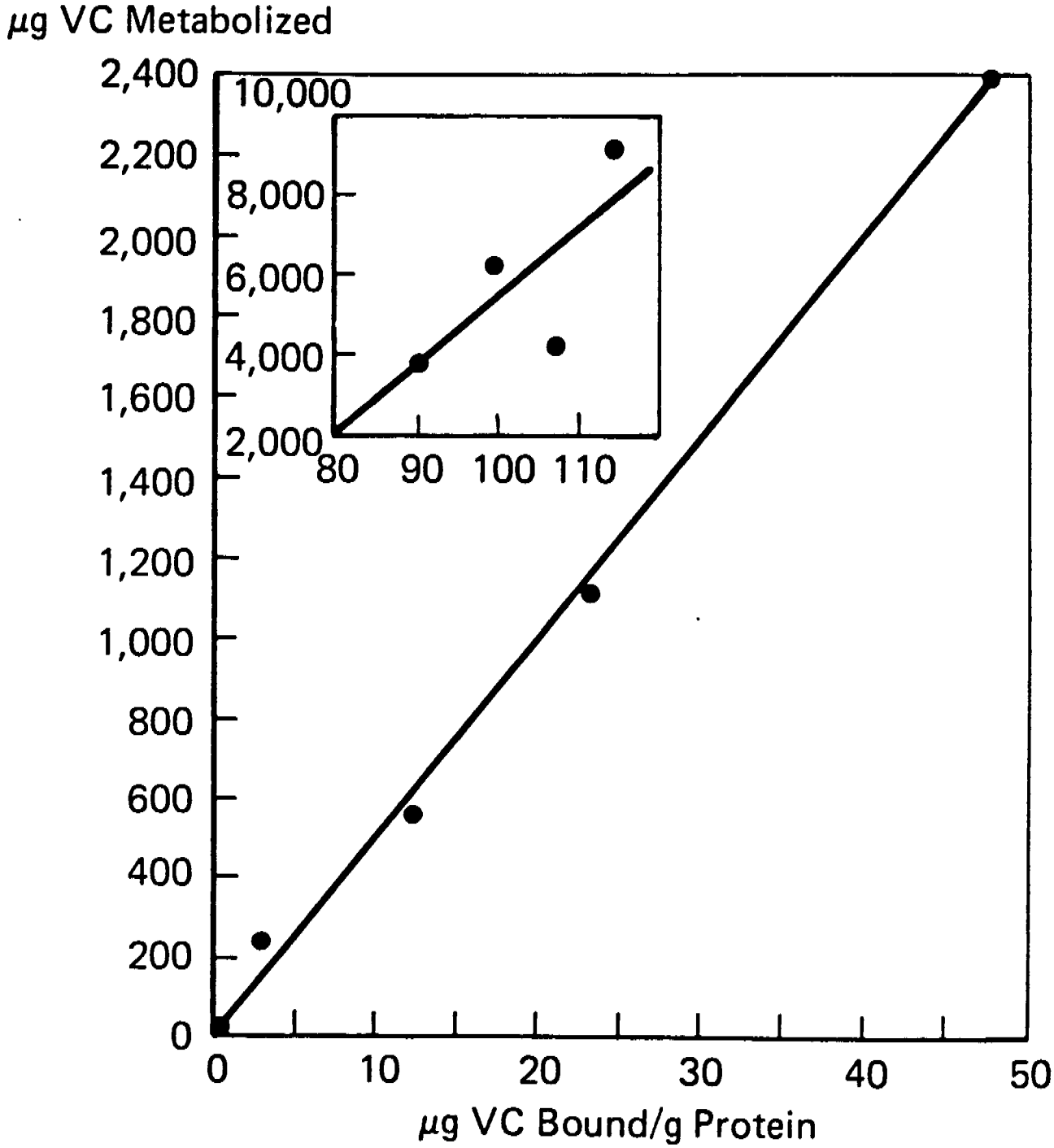


Figure 12