PLAINTIFF'S EXHIBIT 938

J. Med. Res. Journal of Medical Research
Vol 37: 215-223 1917-18

THE ACTION OF BESZOL.[1]

IV. SPONTANEOUS INFECTIONS WITH SPECIAL REFERENCE TO THE DIPHASIC LEUCOPENIA (RABBIT).

H. C. WEISKOTTEN AND H. S. STEENSLAND.
(*From the Department of Pathology, Syracuse University College of Medicine.*)

The four rabbits here considered are of importance, especially, as exhibiting periods of spontaneous infection coinciding with periods of esposure to the action of subcutaneous injections of olive oil-benzol mixture (equal parts of olive oil and benzol). We have included a surgical infection case, which may perhaps be regarded as being on the border line between esperimental and spontaneous infections.

We have shown that under the esperiniental conditions here employed there is constantly produced in non-infected rabbits a diphasic leucopenia.[1,2] In about two days after the beginning of daily injections the leucocyte curve begins to fall and is lowered to about 1,000 in about six days after the beginning of an average of 4.j daily injections with a dosage of 1.5 to 2 cubic centimeters of the mixture per kilo? On the other hand, the most striking feature in connection with the spontaneously infected rabbits is that an average of eight daily injections with a dosage of 2 cubic centimeters of the mixture per kilo failed to initiate and complete this fall in the leucocyte curve in an average of eight days after the beginning of injections. In all except one of the cases the curve even rose in the face of such injections. The infection seemed to be the predominating influence in determining the course of the curve.

In non-infected rabbits it has been noted that the Fall in the leucocyte curve resulting from such injections is accompanied by a marked decrease in the percentage of polynuclear leucocytes, indicating that the polynuclear leucocytes are especially affected after the injections. In these spontaneously infected rabbits, on the other hand, the polynuclear

*Received for publication July 2, 1917.

percentage, instead of falling during continued daily injections, rose in all instances to from eighty-three to ninety-seven before death. At the time injections were begun none of the animals appeared ill and none showed leucocyte counts or polynuclear percentages indicative of the presence of an active acute infection. Yet after the beginning of injections there occurred leucocytosis in all escept one of the cases; in all of the cases, regardless of whether this leucocytosis occurred, the polynuclear percentage showed a decided rise. All of the animals showed a decided loss in weight, two died, and the other two were apparently ill, and were killed. These observations suggest the initiation of a spontaneous acute infection or the " lighting up " of a preëxisting infection after the beginning of injections.

The class of cases considered in this paper came under observation incidentally in connection with other work. It so happens that all of these rabbits were splenectomized for varying periods before receiving injections. We have had no opportunity to make corresponding observations on spontaneously infected non-splenectomized rabbits. However, we have shown1 that in non-infected rabbits the leucocyte curve follows the same course in splenectomized as in non-splenectomized rabbits after injections;

The time of splenectomy in days before- the beginning of injections was as follows: Experiment 14.2, 195 ; Experiment 14.7, 180; Espcriment 14.10, 187; Experiment 14.31, 2. The spleens were fixed at the time of splenectomy in all cases and microscopical examination resulted negatively.

Three of the rabbits had spontaneous infections during all or a part of the period during which injections were being given; two had otitis media, and the third had an infection, the nature of which was not fully determined. One mas found to have post-operative peritonitis, following splenectomy performed two days before injections were begun.

Rabbit Experiment 14.2 (Chart 7) had right otitis media, originating, probably. before injections were begun. Before injections were begun the leucocyte count varied between 8,500 and 15,000. Eight relevant

daily injections wer
show any definite ev
upon :he leucocyte
On the third day
slightly below 6,00
11,000, following wh
reached nearly 23.0
rise occurring in spi



CHA

daily injections were given. The study of the leucocyte curve fails to show any definite evidence of the characteristic effect of these injections upon the leucocyte count. such as that shown by non-infected rabbits. On the third day following the first injection the leucocyte curve fell slightly below 6,000, rising. however, on the following day to nearly 11,000, following which there was a constant or progressive rise until it reached nearly 23.000 on the day on which the animal was killed: this rise occurring in spite of the fact that the daily injections were continued.

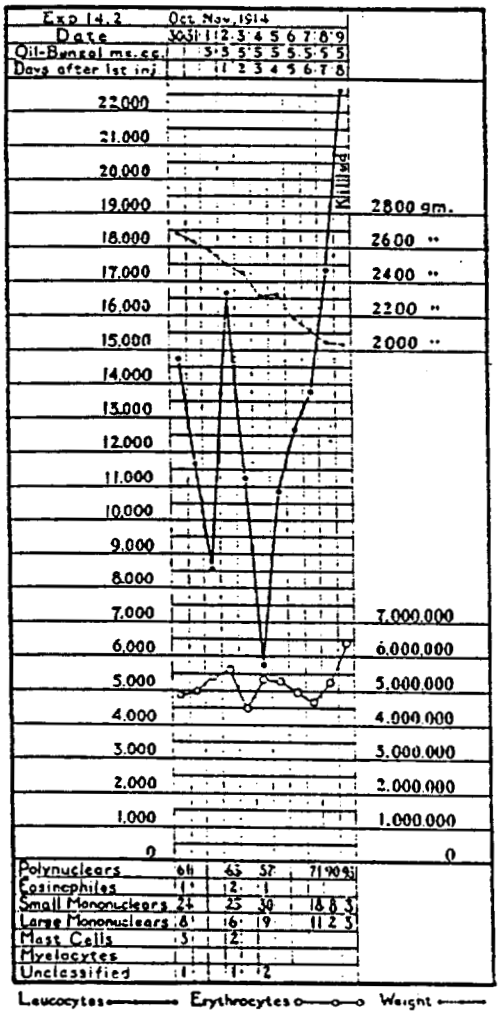


CHART 7 (Erp. 14.2). — Otitis media. **Dosage. 1.9 cubic centimeters** per **kilo.**

Eefore injections were begun tlie polynuclear percentage was in :he neighborhood of sixty. at which level it remained for a number of days after the beginning of injections and up to the last progressive rise. However, during the last progressive rise tlie percentage of polynuclears increased, being ninety-three on the day the rabbit was killed. This definite increase in percentage of polynuclears. aside from being further evidence of an existing infection. is especially noteworthy in view of the fact that injections are usually accompanied by a marked fall in the percentage of polynuclears. Both the leucocyte curve and the differential counts suggest that the injections "lighted up" an already existing infection or led to tlie initiation of an acute infection.

Immediately following the beginning of injections there began a fall in the weight curve which continued until the day tlie rabbit was killed, amounting to nearly 600 grams.

It was regarded as being in qood condition until tlie day after the first injection was given. when the first signs of abnormality in its general condition were noted. On the day it was killed it lay on its side. occasionally moving a leg or its head. and opening its mouth widely every fifteen to thirty seconds.

At autopsy the right middle ear was found filled with a grayish creamy material. smears from which showed numerous bacteria and few pus cells. Sumerous fresh hemorrhages were observed in the mucous membrane of the stomach, and this was confirmed by histological examination.

Examination of the marrow at autopsy showed it to be apparently abundant and of uniform color. Microscopically it was leucoblastic. A few small foci of hemorrhage were seen, but no obvious aplasia. such as occurs in non-infected animals in association with the leucopenia, which mag usually be produced by a much smaller number nf injections.

Rabbit Experiment 14.31 (Chart 8) had post-operative peritonitis following splenectomy. Nothing striking was observed about the clinical symptoms in general. Four daily leucocyte counts preceding splenectomy and the count on the day following splenectomy showed ordinary daily variations, the average being in the neighborhood of 8,500 (polynuclear percentages 33, —, 23, 10. —). Two days after splenectomy, the leucocytes had risen to about 17,600 (polynuclear percentage 61), and on this day injections were begun. Considering the succeeding portions of the curve as a whole. there is no definite evidence that the injections had any lowering effect upon its course : there are great variations in the level of the curve from day to day; the maximum level reached is about 18,600 (polynuclear percentages 44, 37, 6j. 86, 92, 88), and the curve never falls below the original normal level. Seven daily injections were given and the rabbit died at 11 P.M. on the dag of the last injection.

The portion of the weight curve involving four daily weighings during the six days preceding splenectomy shows no variations that could not be explained as a result of normal daily variation. From the dag of splenectomy. it drops rather uniformly from about 2,600 grams on the day

of splenectomy. down to abou
senting a loss of about 750 gra

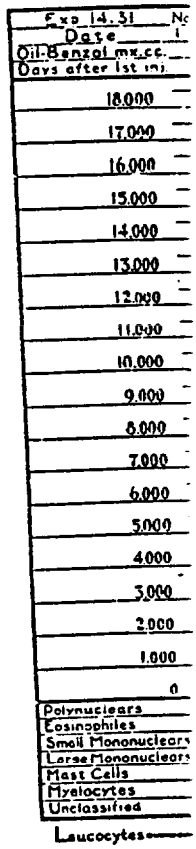


CHART 8 (E:
periton

Examination of the mar
dant and uniformly dee
maker interpretation impo
hemorrhage, marrow cell
nuclears predominate. s
evidence of necrosis an
suggesting aplasia.

There was hemorrhage
with slight local peritoni

of splenectomy, down to about 1,850 grams on the day of death, representing a loss of about 750 grams in eight days.

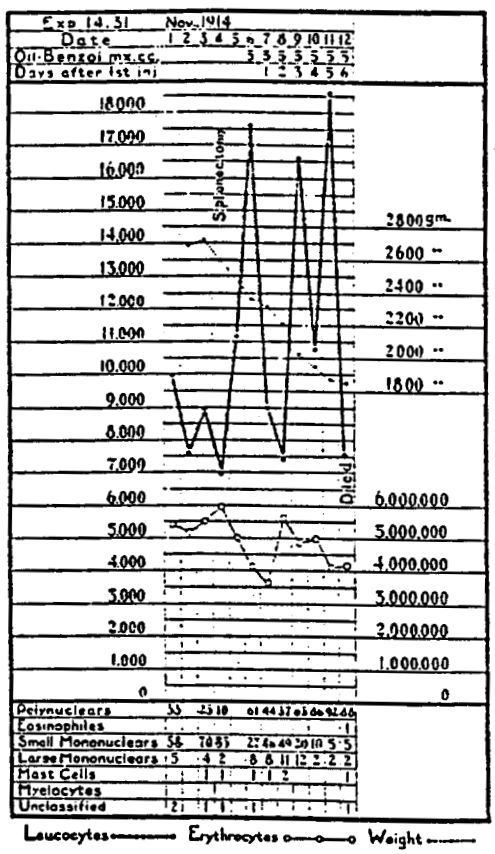


CHART S (Exp. 14.31). — Acute post-operative peritonitis. Dosage, 2.1 cubic centimeters per kilo.

Examination of the marrow at autopsy showed it to be apparently abundant and uniformly deep red. Microscopically, extreme hemorrhage makes interpretation impossible. Apparently, partly at least as a result of hemorrhage, marrow cells appear few; however, myelocytes and polynuclears predominate, suggesting a leucoblastic marrow. There is evidence of necrosis and karyorrhexis in myelocytes and leucocytes suggesting aplasia.

There was hemorrhage into the omentum in the region of the spleen, with slight local peritonitis in the region of hemorrhage, grayish exudate

forming adhesions with intestine. Microscopical examination of the omentum in the region of the exudate showed necrosis and fibrino-purulent exudate.

Rabbit Experiment 14.10 had bilateral otitis media. In this case, evidence of discharge from both ears was observed before the first injection was given. Sine days later at the time the animal was killed. there was a foul discharge from both ears. This animal was rather quiet and was not eating on the day it was killed, but previously had presented no obvious serious symptoms. In three daily counts before injections were given and in fire daily counts after injections were begun, the leucocyte count averaged about 8,000 and showed no variations that might not be accounted for by normal daily variation. During the sixth and seventh days after injections were begun it fell to 3,500. Then. in spite of the fact that daily injections were continued from the beginning, it rose again to 7,500 on the ninth day after injections were begun, when the rabbit was killed.

Two days before injections were begun the polynuclear percentage was thirty-six and there was no material change until the day preceding the above-mentioned fill of the leucocyte curve to 3,joo. when it rose to fifty-two. On the first day of the fall with a leucocyte count of 4,000 the polynuclear percentage was sixty-two; on the second day of the fall with a count of 3,500 it was twenty-eight; at the end of the rise. occurring on the two following days, with a count of 7. joo it was eighty.

The general tendency of the weight curve was downward from the day injections were begun, and nine days after the first injection was given the rabbit had lost about 350 grams.

At autopsy the left middle ear was filled with rather thick brownish gray material, having a foul odor; the right contained a smaller amount of similar material. Smear from left showed numerous bacteria and no pus cells.

The marrow appeared small in volume and uniform in color. Microscopically it was leucoblastic. polynuclears being very numerous. Aside from a few myelocytes or polynuclears in karyorrhexis. no evidence of aplasia was seen. There were a few small foci of hemorrhage.

This case is especially suggestive of the "lighting up" of an already existing infection. inasmuch as discharge from the ears was observed before the beginning of injections. and at that time the general condition of the animal appeared good and the differential count was normal.

Rabbit Experiment 14.7 on the day before death showed a white discharge from one eye. In addition, the leucocyte curve. the differential count, and loss of weight indicated the presence of significant infection. The general condition appeared like that of a non-infected rabbit receiving a like number of injections.

In three daily leucocyte counts before injections were given and two after injections were begun there were no variations that could not be

A(

accounted for by normal d.
(average polynuclear perc
begun the count was 5,2
thus to a typical beginning
polynuclear percentage.
continued. the curve rose
13,750 on the sixth day
centages 69, —. 91). Th
predominate and the curve
tions were begun to a min
day (polynuclear percent
death occurred in the lat
begun.

The case suggests tha
curve may overcome the i
time. As will be noted
However, the polynuclear

From the time injectio
loss of weight, involving
of eight days, the end of

Examination of the m
reddish gray. Microscop
the latter evidenced by
clears, and necrosis. app

Careful and thor
In addition to the
femora, and special
organs were exami
cases: heart, lung,
teric lymph node,
and cervical cord
and Experiment 14.
ment 14.10 and Ex
ment 14.7 and E
Experiment 14.2 ar
cal examination in
enable us to make.
in regard to them,
might be expected

A probable impo
with these rabbits i
ceding death, the

accounted for by normal daily variation, the average number being 8,500 (average polynuclear percentage 45). Three days after injections were begun the count was 5,250 (polynuclear percentage 49), corresponding thus to a typical beginning of a primary fall aside from a moderate rise in polynuclear percentage. Then, in spite of the fact that injections were continued, the curve rose for three days, reaching a maximum level of 13,750 on the sixth day after injections were begun (polynuclear percentages &, —, gr). Then possibly the effect of the injections began to predominate and the curve fell on the seventh and eighth days after injections were begun to a minimum level of 2,500 at the end of the eighth day (polynuclear percentages 97, 93). Without any further injections death occurred in the latter part of the ninth day after injections were begun.

The use suggests that the effect of the injections on the leucocyte curve may overcome the infection effect after this has predominated for a time. As will be noted below, characteristic marrow aplasia occurred. However, the polynuclear percentage remained high.

From the time injections were begun there was a practically continuous loss of weight, involving a total loss of about 500 grams during a period of eight days, the end of the day before the animal died.

Examination of the marrow at autopsy showed it to be uniform dark reddish gray. Microscopically there was marked hemorrhage and aplasia, the latter evidenced by the presence of very few myelocytes, no polynuclears, and necrosis, apparently mainly of myelocytes.

Careful and thorough autopsies were made in all cases. In addition to the spleens, marrow of the shafts of the femora, and special tissues described above, the following organs were examined microscopically in all four of the cases: heart, lung, liver, kidney, pancreas, adrenal, mesenteric lymph node, Peyer's patch, and thyroid. Appendix and cervical cord in Experiment 14.2, Experiment 14.10, and Experiment 14.31. Parathyroid and thymus in Experiment 14.10 and Experiment 14.31. Cerebellum in Experiment 14.7 and Experiment 14.31. Shaft of femur in Experiment 14.2 and Experiment 14.10. Such microscopical examination in all cases showed no changes which would enable us to make any relevant important positive statements in regard to them, but only possible mild degrees of what might be expected in acute infections and toxemias.

A probable important clinical consideration in connection with these rabbits is that on the day of death, or the day preceding death, the leucocyte curves were at relatively high

levels. In connection with the therapeutic use of benzol it

I. During- daily
benzol mixture in fou
active acute infectio
before injections were

2. The results ind
infections present
"lighted up" after
that injections may r
less quiescent or late

3. In none of tht
penia and in only o
produced in similarl

4. Under the cc
cyte count is not a e
istration of benzol.
of this fact upon th

1. Weirkotten, H. G
action of benzol. I.
spleen. Jour. Xed. Re

2. Weiskotten, H. C
action of benzol. II.
antigen-mubody react

3. Reference No. 1,

4. Rusk, G. Y. St
effect of benzol intoxic
of artificial hemolysi
1914, ii, 139.

5. Hektoen. Ludv
antibodies. Jour. Inf

CONCLUSIONS.

1. During daily subcutaneous injections of olive oil-benzol mixture in four rabbits there developed evidences of active acute infection, which evidences were not present before injections were begun.

2. The results indicate that in at least two of the rabbits infections present before injections were begun were "lighted up" after injections were begun. This suggests that injections may result in the "lighting up" of more or less quiescent or latent infections.

3. In none of the rabbits did there occur diphasic leucopenia and in only one aplasia of the marrow, such as are produced in similarly treated non-infected rabbits.

4. Under the conditions of our experiments the leucocyte count is not a safe guide in connection with the administration of benzol. We wish to suggest a possible bearing of this fact upon the therapeutic use of benzol.

REFERENCES.

1. Weiskotten, H. G., Schwartz, S. C., and Steensland, H. S. The action of benzol. I. On the significance of myeloid metaplasia of the spleen. Jour. Med. Research, 1915, xxxiii, 127.

2. Weiskotten, H. G., Schwartz, S. C., and Steensland, H. S. The action of benzol. II. The deuterophase of the diphasic leucopenia and antigen-antibody reaction. Jour. Med. Research, 1916, xxxv, 63.

3. Reference No. 1, Table I., and Charts 1, 2, and 3.

4. Rusk, G. Y. Studies on the locus of antibody formation. II. The effect of benzol intoxication and consequent leucopenia on the formation of artificial hemolysins and precipitins. Univ. Calif. Publ. Pathology, 1914, ii, 139.

5. Hektoen, Ludvig. The effect of benzene on the production of antibodies. Jour. Infect. Dis., 1916, xix, 69.