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Liver Extract and Reticulocytosis in the Guinea Pig.

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Jacobson[1] has reported that an increase in the number of circulating reticulocytes follows the intraperitoneal injection in the guinea pig of liver extract which is curative of pernicious anemia. The results of this test were stated to be sufficiently accurate to differentiate not only between potent and non-potent liver extracts, but even to quantitate the antipernicious effect of various dilutions of the same extract. Goodman, Geiger, and Klumpp[2] have failed to confirm this observation. They stated that guinea pigs showed an irregular reticulocytosis without treatment and that no response to the injection of antianemia substances occurred.

Miller and Rhoads[3] published studies based upon Jacobson's method and concluded that an increase of guinea pig reticulocytes followed the feeding of various antianemia substances. This method avoided the possible non-specific reticulocytogenic effect of the intraperitoneal injections of irritant substances.

In view of the very striking discrepancy between the experimental results of Jacobson and of Goodman and his coworkers it was thought advisable to reinvestigate the subject in an attempt to obtain an answer to the following 5 questions:

1. Do spontaneous variations in the number of circulating reticulocytes occur in guinea pigs maintained under absolutely constant conditions? 2. If spontaneous variations occur are they of such a degree and frequency as to serve as a valid objection to the employment of the guinea pig as a test animal? 3. Does the guinea pig with otherwise constant reticulocyte levels react to the oral administration of potent liver extract by a reticulocytosis? 4. Does the oral administration of the same liver extract after inactivation by heat provoke similar responses? 5. Do factors other than the administration of liver extract cause reticulocytosis in the guinea pig?

Experiments bearing on these 5 fundamental points have been made. The percentage of reticulocytes of normal guinea pigs, kept

[1] Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 665.

[2] Goodman, L. S., Geiger, A. J., and Klumpp, T. G., *J. Clin. Invest.*, 1936, **15**, 435.

[3] Miller, D. K., and Rhoads, C. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 419.

under standard conditions, have been followed daily for 6 months. The effects of changes in diet, of liver extract feeding, and of feeding liver extract after inactivation of the anti-anemia factor have been studied.

Nineteen adult white guinea pigs of the same strain were kept under absolutely the same conditions of room, cages, bedding, food, and handling as far as was possible. Reticulocytes were counted by the dry-smear method. The animals were bled by pricking a minute ear vein. The greatest care was observed to allow no more than 0.05 cc. of blood to escape. Smears were made by the usual method on cover slips upon which a film of brilliant cresyl blue had been dried. Many check counts gave very few divergent results. As a source of anti-anemia substance, liver extract, Lilly N.N.R., was employed in a 50% solution in water. It was fed from a pipette. Inactivated liver extract was prepared according to the method of Goodman by heating to 200° for 2 hours in a chemical oven.

During the first 2 weeks of observation the levels of reticulocytes varied considerably without treatment and in a number of the animals reached heights which would be considered positive response to therapy according to the criteria of Jacobson. The diet was changed after 17 days of study after the reticulocytes had been stable for 7 days. At this point 2 guinea pigs had been discarded because of intercurrent disease. In 9 of the remaining animals a distinct reticu-

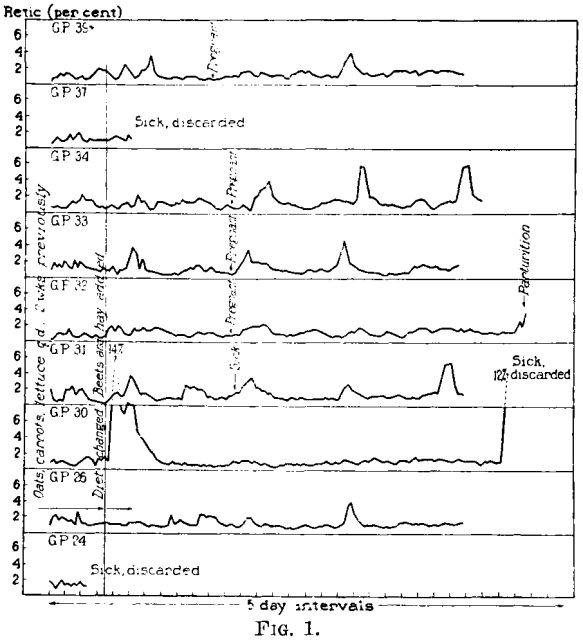

FIG. 1.

locytosis occurred following the change in diet, whereas in 8 no such effect was observed. During the next 3 months 7 more guinea pigs were discarded, 4 because of pregnancy with reticulocytosis, 2 with intercurrent disease and reticulocytosis, and 1 with reticulocytosis without apparent cause. (Fig. 1.)

Experiment I. (Fig. 2.) From the original group 10 animals remained which had at no time during 3 months of constant observation shown any increase in reticulocytes to levels of 2% or over for a period of 2 successive days. When these 10 guinea pigs were fed 0.6 gm. daily of liver extract, Lilly, every animal showed an increase in numbers of reticulocytes which was sustained, was well above the 2% level, which came within the time limits of a reticulocyte rise in human pernicious anemia, and which showed a so-called physiological curve with a rise and then a fall. Such consistent results would seem to be wholly outside of the range of either spontaneous rises or of experimental error.

Experiment II. (Fig. 2.) The 10 animals which had responded by reticulocytosis to the feeding of liver extract were fed the same amount of liver extract after inactivation by charring according to the method of Klumpp. No increase of reticulocytes was observed.

Experiment III. After a failure to respond to inactivated liver

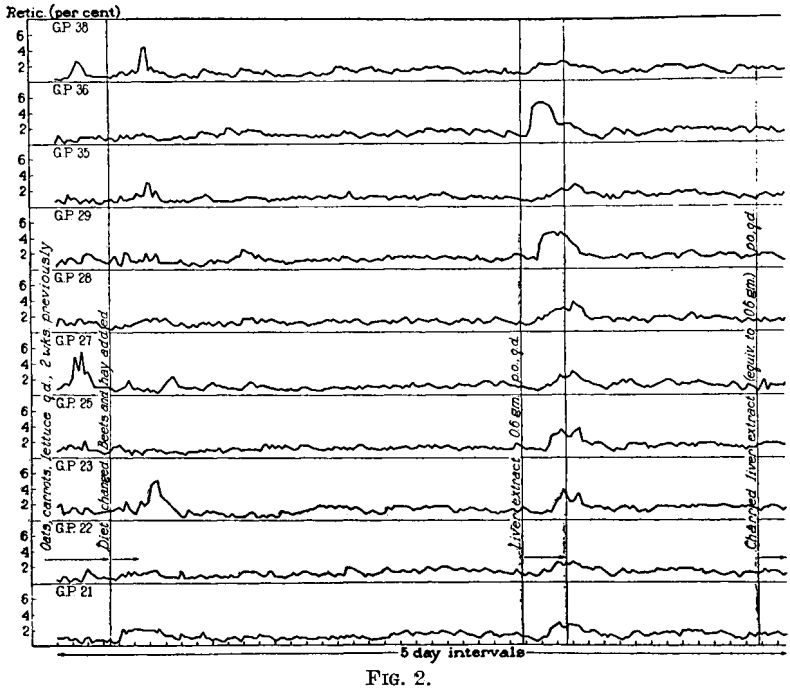

FIG. 2.

extract the same animals were retested by feeding once more liver extract of known potency. For a second time all the animals responded with a reticulocyte peak although not so dramatically as after the first test.

From the experiments presented it appears that increases in reticulocyte numbers can follow a variety of events. Changes in dietary, pregnancy, injury, and infection all seem to exert an effect. Jacobson[4] has shown that excessive loss of blood may also be productive of rises. Lastly, rises do take place rarely, which are spontaneous as far as can be ascertained. It is also clear that animals can be selected which show no serious variation in reticulocyte numbers over a 3-month control period under absolutely constant conditions. These same animals will respond to the oral administration of liver extract by a rise in reticulocytes and fail to respond to inactivated liver extract.

When these experiments are compared to those of Jacobson and of Goodman, two sharp differences are immediately apparent. Our animals were tested by the oral administration of liver extract, both active and inactivated. This route of administration was selected in order to avoid the possible error inherent in any method which depends upon the injection of a known irritant substance. Any error which might occur from the use of the oral as opposed to the parenteral route should clearly be on the side of failure to respond rather than of false positive responses.

The second discrepancy between the results of our experiments and those of other workers concerns the use of very long control periods (3 months) and the rigid exclusion of any animals which evince a rise of reticulocytes from whatever cause during those periods. From the rarity of spontaneous rises in our experiments it is clear that they should not be a serious factor in the interpretation of results from animals which are kept under absolutely standard and constant conditions. Our experiments cannot be compared justly to those of either Goodman or Jacobson however, since in the studies of both of them short control periods were employed; hence, periods of handling and bleeding alternated with periods of rest. In such a delicate mechanism as the maintenance of reticulocyte levels it is inevitable that such factors could easily increase the numbers of reticulocytes by one per 100, sufficient to give a response by Jacobson's criteria.

It is not the purpose of these experiments to answer the question of whether or not the guinea-pig response is due to the administra-

[4] Jacobson, B. M. Personal communications.

tion of that substance which is effective in the induction of remissions in pernicious anemia of human beings. Their only aim is to show that under proper conditions reticulocytes may remain at constant levels and that rises occur when liver extract is fed. Liver extract is a complicated and little-understood substance. It is rich in the vitamin B complex as well as in the anti-anemia factor. The question of which constituent is effective in the guinea pig must await further investigation.

Summary and Conclusions. 1. The number of circulating reticulocytes in the guinea pig increases after a variety of physiological disturbances. 2. By suitable care in handling and feeding stable reticulocyte levels may be maintained for as long as 3 months. 3. Rises of reticulocytes follow the feeding of liver extract. 4. Similar rises do not follow the feeding of charred liver extract.

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Effect of Colchicine on Human Tumors.

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The action of colchicine upon division of normal and malignant cells has been studied by several investigators. Dustin[1] described a remarkable increase in the number of mitoses in malignant growths in animals following the injection of colchicine. Ludford[2] found that colchicine applied to cultures of normal and malignant cells of animals brought about an arrest of mitosis for several hours, and that prolonged application destroyed the cells held up in division while the resting cells still survived, and that a single injection in animals produced an arrest of mitosis for several hours. The mitoses were arrested in the metaphase, which Ludford believes is due to the failure of the mitotic spindle to form and function in the normal manner. He also found that the largest accumulation of arrested mitoses occurred in tissue in which cell division is normally of frequent occurrence. This phenomenon has been utilized by others[3, 4] as an index of the rate of cell growth. It, therefore,

[1] Dustin, A. P., *Bull. Acad. Med. Belg.*, 1934[2], **14**, 487.

[2] Ludford, R. J., *Arch. f. exper. Zellforsch.*, 1936, **28**, 411.

[3] Brues, A. M., *J. Physiol.*, 1936, **86**, 63.

[4] Allen, Edgar, Smith, G. M., and Gardner, W. U., *Endocrinology.* In press.