accessory and selective pathways, if existing at all, would seem to be of minor significance in relation to the amount metabolized along the conventional pathways.

Summary. Pairs of mice were administered either by mouth or by injection equal amounts of ethanol-1-C^{14} or acetate-1-C^{14}. The phospholipid, non-saponifiable fraction, and fatty acid fractions of liver, gut, brain, and fat were isolated and specific activities of these fractions were compared. Ethanol-1-C^{14} and acetate-1-C^{14} contributed approximately equal labelling to tissues investigated.

1. Schulman, M. P., Zurek, R., Westerfeld, W. W., in Himwich, H. E., ed. *Alcoholism, Basic Aspects and Treatment;* 29-37. Washington, D. C., 1957, Publ. No. 47.

2. Sinclair, R. G., Dolan, M., *J. Biol. Chem.,* 1952, v142, 659.

3. Bernhard, K., *Z. Physiol. Chem.,* 1940, v267, 99.

4. Bloch, K., Rittenberg, D., *J. Biol. Chem.,* 1944, v155, 243.

5. Richert, D. A., Westerfeld, W. W., *ibid.,* 1957, v227, 533.

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Inhibition of Neutrophil Mobilization by Colchicine.* (25809)

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Colchicine is a powerful mitotic poison reported to cause extensive bone marrow damage, arresting division of both developing leukocytes and immature erythrocytes(1). In contrast to the depressive effects upon bone marrow, colchicine injection provokes a short-lived leukopenia followed by a marked increase in numbers of circulating granulocytes in the blood of dogs, rabbits, and mice(2,3). The relationship between bone marrow inhibition and peripheral blood leukocytosis is a puzzling one and remains to be elucidated. Therefore, it was felt that it would be of interest to investigate another parameter of this problem, namely ability of the animal to mobilize neutrophils following colchicine treatment.

Methods. Male rats of the Sprague-Dawley strain and weighing 200-240 g were used. All experimental rats received a single, subcutaneous injection of 0.2 mg colchicine (Lot 920 LPA, S. B. Penick Co.) dissolved in 0.2 ml of 0.9% saline; control animals received 0.2 ml of 0.9% saline. Immediately following subcutaneous injection and at 24, 48, 72 and 96 hours thereafter, groups of 6 animals were challenged by an intraperitoneal injection of 1 μg Piromen,† a Pseudomonas polysaccharide complex (Baxter Lab.) dissolved in 10 ml 0.9% sterile, nonpyrogenic saline. These injections were administered to animals while they were under light ether anesthesia. The rats were sacrificed 5 hours after intraperitoneal injection of bacterial endotoxin. Leukocytes in the peritoneal fluid were harvested and their numbers and types determined from hemacytometer counts and from Wright-Giemsa-stained smears. Previous work(4,5) had established the efficacy of this treatment in evoking a marked neutrophilia in peritoneal fluid of normal, untreated rats.

Results. Fig. 1 summarizes the effects of colchicine upon the pattern of neutrophil mobilization at various times following its injection. Rats challenged by an intraperitoneal injection immediately following injection of colchicine revealed a drastic reduction in numbers of neutrophils harvested from the peritoneal fluid 5 hours later. Such fluid contained less than 8 million neutrophils in contrast to over 59 million neutrophils that were obtained from non-colchicinized, control animals. Twenty-four hours after colchicine injection, challenged rats mobilized neutrophils

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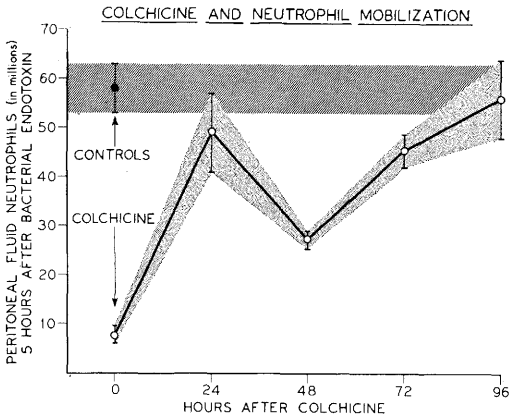

FIG. 1. Effects of a single inj. of colchicine upon neutrophil mobilization into peritoneal fluid of rats induced by inj. of bacterial endotoxin. Each circle represents mean value for 6 rats. Vertical lines represent ± 1 stand. error of mean.

in a manner comparable to the controls, but at 48 hours fewer cells than normal were mobilized. This was still somewhat the case at 72 hours. However, the normal pattern of mobilization was eventually restored since animals challenged 96 hours after colchicine treatment responded by massing normal numbers of neutrophils into the peritoneal fluid. Morphologically, the neutrophils in all the groups tested appeared to be of the mature variety, resembling those seen in circulating blood of untreated rats. Peripheral white blood cell counts revealed a transient leukopenia which was followed by a marked increase in circulating neutrophilic granulocytes.

Discussion. The most striking inhibition of neutrophil mobilization occurred in animals challenged immediately after injection of colchicine. It is unlikely that the anti-mitotic effects of colchicine played a crucial role in this phenomenon in view of the tremendous reserve of neutrophils possessed by the rat (6) and the rapidity with which this inhibition developed. Furthermore, the depression in numbers of mobilized neutrophils was seen at a time when similarly-treated animals have been reported to display a peripheral leukocytosis(2,3). There is evidence that colchicine can exert a variety of actions that apparently are not related to its effects upon mitosis. For example, colchicine has been shown to alter the morphology of the mast cell(7) as well as having effects on the nervous system, muscle and vascular system(1). Of direct interest to the problem in hand, are the findings that epinephrine(8), large doses of bacterial endotoxins(9) and hemorrhagic shock(10) also have the ability to inhibit diapedesis of neutrophils. It is possible that all of the aforementioned treatments as well as the effects noted following colchicine may act *via* a common pathway which affects the vascular system.

A second reduction in mobilization of neutrophils was observed in rats that had been challenged by endotoxin 48 hours after being injected with colchicine, and this response did not revert to normal until 96 hours after injection. This secondary loss of ability on the part of the animal to mobilize neutrophils may be a reflection of the well-known anti-mitotic and destructive actions of colchicine upon bone marrow.

The importance of neutrophil mobilization as a defense mechanism during the first few hours following bacterial invasion has been described(8). It is felt that the use of bacterial endotoxins to stimulate an influx of neutrophils, as in the present experiments, mimics, in many ways, the response to an actual infection. Thus, the possibility must be considered that, under certain conditions, colchicine treatment may render the organism more susceptible to bacterial infection. This depressive effect of colchicine upon neutrophil mobilization is of more than academic interest, since this drug is widely used in treatment of gout while its mode of action in this disease is still a mystery(11).

Summary. A single injection of colchicine markedly inhibited ability of rats to mobilize neutrophils. The greatest inhibition was seen when animals were challenged immediately following colchicine injection. A secondary inability to mobilize neutrophils appeared at 48 hours and was still present at 72 hours following colchicine treatment. The significance of these phenomena is considered.

1. Eigsti, O. J., Dustin, P., Jr., *Colchicine—In Agriculture, Medicine, Biology, and Chemistry,* Iowa State Press, Ames, 1955, pp175-201.

2. Dixon, W., Malden, W., *J. Physiol.,* 1908, v37, 50.

3. Widmann, H., *Arch. Exp. Path. u. Pharm.,* 1949, v207, 218.

4. Fruhman, G. J., *Experientia,* 1959, v15, 226.

5. ———, PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 423.

6. Donohue, D. M., Reiff, R. H., Hanson, M. L., Betson, Y., Finch, C. A., *J. Clin. Invest.,* 1958, v37, 1571.

7. Padawer, J., *Trans. N. Y. Acad. Sci.,* 1957, v19, 690.

8. Miles, A. A., *Lectures on the Scientific Basis of Medicine,* pp235-258, U. of London, Athlone Press, 1955.

9. Delaunay, A., Pages, J., *Ann. de l'Inst. Pasteur,* 1945, v71, 431.

10. Rutenburg, S., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 217.

11. Weiss, T. E., Segaloff, A., *Gouty Arthritis and Gout,* 221 p, C. C. Thomas, Springfield, Ill., 1959.

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Response of Mouse Breast Tumors to L-Triiodothyronine and Irradiation. (25810)

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In 1951 it was shown that mice made hyperthyroid by oral administration of thyroxine showed increased mortality when given total body irradiation(1). A year later L-triiodothyronine (T3) was isolated from human plasma(2). T3 by various assays was several times as active as thyroxine, and it was postulated that thyroxine is deiodinated to T3 in peripheral tissues before exerting its effect(3). In animals peak physiologic activity with increased oxygen consumption was exhibited by fourth day of T3 administration(4). L-triiodothyronine was strongly accumulated by Ehrlich mouse ascites carcinoma cells(5). Increased glycolysis without increased mitotic rate of growing tumor cells in tissue culture was observed after treatment with T3(6). Stein and Griem studied the effect of combined T3 and x-ray therapy on myeloid chloroleukemic rat tumor, mouse neuroblastoma, and human bronchogenic carcinoma(7). They noted more marked tumor regression after combined therapy so that a lower x-ray dose would accomplish the same therapeutic result. The skin reaction was accelerated. The purpose of this study was to evaluate the effect of x-radiation on transplanted mouse breast adenocarcinoma when animals had been made hyperthyroid by administration of L-triiodothyronine.

Method. Randomly selected ZxC57 black F_1 male and female hybrid mice, aged 3 to 6 months, were kept in cages in air-conditioned room maintained at 72°F. An ample supply of tap water and Purina fox chow was always available. Twenty-five mg of T3 were dissolved in 25 cc of propylene glycol and 25 cc of sterile water and kept in freezing compartment. The same material was used throughout preliminary toxicity studies as well as actual experiment. Five groups of 5 mice each were injected intraperitoneally with .25, .50, .75, 1, and 2 mg/kg respectively, for 4 consecutive days. Weights were recorded daily and animals observed for signs of hyperthyroidism. The first 3 groups developed moderate diarrhea and a transient weight loss, most marked on third day. Weights were back to normal by 6th day. In groups receiving 1 mg/kg weight fluctuation was less marked but signs of hyperthyroidism were evident. The animals had diarrhea, loss of coat sheen, marked increase in activity, increased water and food consumption without increase in weight. The group receiving 2 mg/kg showed polydipsia, transient weight loss, bulimia, increased respiratory rate, and loss of coat sheen. By 5th day these animals showed extreme lassitude. Dose of 1 mg/kg was optimum and this was in the dose range employed by Stein and Griem(7). Spontaneous adenocarcinoma of the breast originating in a Z female mouse and transferred by serial transplantation for 2 generations was processed