of affected tissues. Apparently the azo dyes are absorbed from the gut into the hemolymph and are then taken up by the Malpighian tubules. If the tubules cannot excrete the dyes or their degradation products into the hind-gut, as is ordinarily done with other excretory substances,[9] the dyes or their derivatives will accumulate in the proximal portion of the tubules. Occlusion of the open ends of the tubules could lead to their swelling into vesicles (Fig. 2) as the absorption of fluid by the tubules continues. If the ends remain open, but the dyes stay within the tubules, local effects of the dye could cause the tubules to degenerate, become sticky and adhere to adjacent tubules.. Hemocytes then accumulate around the affected areas to form nodules (Fig. 2 and 3). Wigglesworth[10] cites similar cases in which the hemocytes of insects encapsulated internal parasites, or walled off areas of infection or irritation. An alternative explanation of nodule formation would be that the nodules are tumors which were formed by the action of the azo dyes upon the Malpighian tubules. However, there appears to be no evidence at present which would lend support to this view.

Summary. The azo dyes *p*-dimethylaminoazobenzene (DAB), m'-methyl-*p*-dimethylaminoazobenzene (m'DAB) and aminoazobenzene (AAB) were fed to the roach *Blattella germanica* (L) at varying levels in both crude and synthetic diets. A dietary level of 0.2% of the dyes had little or no effect upon growth rate, survival or reproduction of the insects. However, characteristic lesions at the proximal ends of the Malpighian tubules occurred in roaches fed the dyes. DAB was the most active, m'DAB was next, and AAB was least active of the compounds fed.

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10 Wigglesworth, V. B., Insect Physiology, London, 1942, 236.

17194. Relation of Pantothenic Acid to White Blood Cell Response of Rats Following Stress.*

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Recent work has emphasized the role of the adrenal cortex in controlling the number of lymphocytes in the circulating blood following various forms of stress. Corticosterone produced a lymphopenia when injected into either adrenalectomized or intact mice.[1] The injection of the adrenocorticotropic hormone (ACTH) of the pituitary also produced a lymphopenia, provided the adrenals were present.[1,2] A deficiency of pantothenic acid causes lipid depletion and necrosis of the adrenal cortex.[3-6] Because of the importance of pantothenic acid to the physiological integrity of the adrenal cortex and in view of the influence of the adrenal cortex on the number of white blood cells, experiments have been done to determine the effect of diets adequate and deficient in pantothenic acid on the response of the white cells of rats subjected to stress.

Experimental. Young male rats of the Long-Evans strain, bred in the laboratory,

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TABLE I.
Supplements Added to 100 g Basal Diet (22% Vitamin-free Casein, 64% Sucrose, 9% Primex, and 5% NaCl-free Salt Mixture).

	Diet 100	Diet 1	Diet 14
Cod liver oil	1.9 ml	1.9 ml	1.9 ml
d, l-α-tocopherol acetate	145 mg	.0 "	.0 "
Thiamine chloride	1.0 "	.3 mg	.3 mg
Pyridoxine chloride	1.0 "	.3 "	.3 "
Nicotinic acid	10 "	.0 "	.0 "
p-Aminobenzoic acid	30 "	.0 "	.0 "
Riboflavin	2.0 "	.9 "	.9 "
Inositol	5.0 "	.0 "	.0 "
Choline chloride	100 "	.0 "	.0 "
Biotin	.05 "	.0 "	.0 "
Pteroylglutamic acid	.20 "	.0 "	.0 "
Calcium pantothenate	1.0 "	.0 "	43.6 "

were used. They were fed one of the 3 diets whose composition is shown in Table I. Diet 1, the pantothenate deficient diet, and Diet 14, the high pantothenate diet, have been used in previous experiments reported from this laboratory.[5,7,8] Diet 100 is a modification of a diet used by Emerson.[9] The rats consumed 10-15 g of Diets 14 and 100, and 6-10 g of Diet 1 daily.

Experiment 1. The response to stress of normal rats on a complete diet containing an adequate[10] but minimal quantity of calcium pantothenate was studied. Rats 80 days of age were placed on Diet 100 *ad lib.* and 1% NaCl as drinking water for 2 weeks. At the end of this time they were subjected to swimming in large stone crocks filled up to 4 inches of the top with tap water at 25°C. The rats were obliged to swim for 25 minutes. Just before swimming and at 2, 4, and 6 hours afterwards, tail blood was taken for total leucocyte counts and for blood smears. The total leucocytes were counted in the usual manner; estimation of the per cent lymphocytes was based on differential counts of at least 200 cells. The white blood cell response was studied in rats fasted for 17 hours before swimming and in rats allowed food up to the time of swimming. All animals were provided with food after swimming.

Experiment 2. Male rats 30 days of age were placed on the pantothenate deficient diet (Diet 1) and were given 1% NaCl as drinking water. After 25-30 days on the deficient diet, the animals were swum under the same conditions as the rats in Experiment 1 and the response of the white blood cells was followed.

Experiment 3. After 30 days on the deficient diet the rats were transferred to Diet 14, which provided them with about 4 mg of calcium pantothenate daily. After 4-12 days on this diet the stress experiments were repeated and the response of the white blood cells observed.

Experiment 4. Normal rats on Diet 100 and pantothenate deficient rats on Diet 1 were given 4 mg ACTH† per 100 g body weight intraperitoneally. Immediately before the injection of ACTH and at 1, 2, 3, 4, 6 and 8 hours afterward the total leucocytes and percentage leucocytes were determined as before. The animals were not fasted for this experiment.

Results. The response of the white blood cells following swimming or ACTH in the rats on the various diets is shown graphically in Fig. 1. The figures on the left summarize experiments in which food was allowed up to the time of stress, and on the right the response following a period of fasting is shown. Each point represents the average of counts on 12-21 rats.

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[8] Dumm, M. E., and Ralli, E. P., *Endocrinology*, 1948, **43**, 283.

[9] Emerson, G., personal communication, 1948.

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† The ACTH used in these experiments was supplied by the courtesy of Dr. John Mote of Armour and Company.

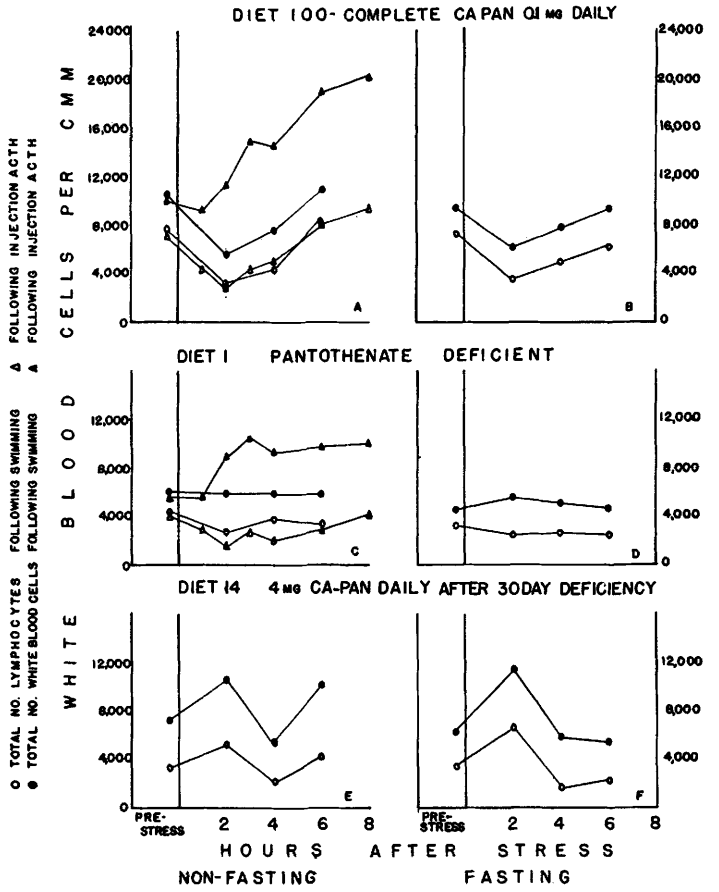

FIG. 1.
Effects of stress on response of white blood cells in rats on diets deficient in and supplemented with pantothenic acid.

The results of the experiments on normal rats maintained on Diet 100 are shown in Figure 1, A and B. This diet provided about 0.1 mg of calcium pantothenate daily. The experiments illustrate the marked lymphopenia which is known to occur when normal rats are subjected to stress.[1] The lymphocyte response was the same after swimming as after the injection of ACTH. Two hours after swimming or ACTH the lymphocyte counts had decreased to about half the initial values. After 6 hours the number of cells had returned to their initial values. The total leucocytes also decreased following swimming, but increased to about twice their initial value following ACTH. The results of the swimming experiment with fasted normal rats (Fig. 1, B) were similar to those in fed rats, although the initial counts were slightly lower.

The results in the pantothenic deficient rats are shown in Fig. 1, C and D. The total number of white blood cells and lymphocytes was lower in these rats than in the rats on the adequate diet. Both swimming and the injection of ACTH were followed in these animals by a small, but probably significant ($p < .05$), decrease in lymphocytes after 2 hours. The lymphocyte response was more pronounced after ACTH than after swimming. After ACTH the total number of white blood cells increased to about twice their original level but there was no significant change in the total number of white blood cells after swimming in the fed animals and only a small increase in the fasted rats.

Discussion. The data presented show that pantothenic acid can modify the response of the white blood cells in rats subjected either to a natural form of stress or to ACTH. In rats on normal diets both swimming and ACTH were followed by a significant decrease in the number of lymphocytes, occurring within 2 hours. In rats that had been fed a diet deficient in pantothenic acid for 3-4 weeks the lymphopenia following stress was largely abolished. Lipid depletion of the fascicular and reticular zones of the adrenal cortex has been reported after a period of pantothenic acid deficiency[5,6] and these are the zones probably responsible for the secretion of the corticosterone-like substances.[11] Dougherty and White[1] have shown that the injection of corticosterone in mice will produce lymphopenia. It may well be that the lipid depletion of the adrenal cortex produced by pantothenic acid deficiency was responsible for the decreased lymphocyte response to stress in the rats.

The addition of very large doses of calcium pantothenate to the diet of deficient rats restored the white blood cell response to stress. The nature of the response under these conditions was not that of the normal rat. The lymphopenia was delayed until the 4th hour after stress and was preceded by an increase in the lymphocytes at the 2nd hour. This occurred in both the fasted and non-fasted rats.

While general malnutrition is associated with adrenal cortical changes, it seems unlikely that this was an important factor in the results presented here. Deane *et al.*[6,12] have reported that neither pyridoxine nor riboflavin deficiency produced lipid depletion in the adrenal cortex of the rat and that inanition alone did not exhaust the adrenal as rapidly as a deficiency in either pantothenic acid or thiamine.

The decrease in the response of the white blood cells to stress in the animals on diets deficient in pantothenic acid is consistent with the changes in the adrenal cortex associated with the deficiency and is further evidence that pantothenic acid plays an important role in maintaining adrenal cortical function.

Summary. The responses of white blood cells and lymphocytes following swimming and following ACTH were compared in rats on a complete diet, in rats on a pantothenic acid deficient diet, and following swimming only in rats recovering from a period of pantothenic acid deficiency. A typical lymphopenia occurred 2 hours after either swimming or ACTH in rats on the complete diet. This response was partially abolished following either swimming or ACTH in the rats on the pantothenate deficient diet. In rats recovering from pantothenate deficiency, there was an increase in lymphocytes 2 hours after swimming followed by a decrease at 4 hours. These results are interpreted as a reflection of the changes in the adrenal cortex induced by pantothenic acid deficiency.

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