ating and following changes in the activity status of rheumatic patients. The present study has been limited to children, and the criteria set forth may not be applicable to adults. There are indications that readings in adults may require more than the 30 minutes we have established for final readings in children. Concentration of the ointment may be a factor.

Many more subjects will have to be studied before final evaluation can be made as to the diagnostic value of skin testing with the tetrahydrofurfuryl ester of nicotinic acid in rheumatic fever. Studies in rheumatoid arthritis and collagen vascular diseases are also indicated. The mechanism of this reaction, as well as the possible role of beta hemolytic streptococcal infection, are under investigation.

Summary. 1. Inunction with an ointment containing 5% tetrahydrofurfuryl ester of nicotinic acid (Trafuril) in 337 children caused localized cutaneous hyperemia and/or edema ("typical" reaction) in normal subjects and those with congenital heart disease or non-rheumatic conditions. 2. Twenty of 22 patients with active rheumatic disease failed to show this typical hyperemia or edema. Their response ("atypical") consisted of either blanching, failure to react, or barely perceptible hyperemia. 3. Inactive rheumatics and patients whose activity was suppressed by cortisone or aminopyrine gave typical reactions. 4. The atypical response was observed in tonsillitis and streptococcal sore throat, as well as rheumatic activity.

1. Jones, T. D., *J.A.M.A.,* 1944, v126, 481.
2. Rothbard, S., Watson, R. F., Swift, H. F., and Wilson, A. T., *Arch. Int. Med.,* 1948, v82, 229.
3. Swift, H. F., *Ann. Int. Med.,* 1949, v31, 715.
4. Waksman, B. H., *Medicine,* 1949, v28, 143.
5. Kahn, L., and Goldring, D., *J. Missouri State Med. Assn.,* 1952, v49, 33.
6. McCarty, M., *Ann. Int. Med.,* 1952, v37, 1027.
7. Nassim, J. R., and Banner, H., *Lancet,* 1952, v1, 699.
8. Gross, F., and Merz, E., *Schweiz. med. Wchnschr.,* 1948, v78, 1151.
9. Strehler, E., *ibid.,* 1949, v79, 144.
10. Gatzek, H., and Mechelke, K., *ibid.,* 1950, v80, 1341.
11. Stark-Mittelholzer, O., *Dermatologica,* 1950, v100, 23.

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Influence of Diet on Serum Alkaline Phosphatase in Rats and Men. (20735)

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The determination of serum alkaline phosphatase has proved to be valuable in the diagnosis of certain diseases of the bones and liver. There is no satisfactory explanation for the phenomena on which these tests are based. Kay(1) suggested that bone was the major source of the enzyme. Gould and Schwachman(2) came to similar conclusions from their experiments on scorbutic guinea pigs. Armstrong and Banting(3) found that removal of viscera in animals did not affect the serum phosphatase level and therefore, concluded that most of the serum phosphatase came from bone. The work of Bodansky(4) on serum phosphatase of dogs under various dietetic conditions is significant. He found that prolonged fasting greatly reduced the serum phosphatase activity of young dogs and that an increase of this enzyme was obtained only after ingestion of carbohydrates. Ingestion of cream or meat did not have any effect. Weil and Russel(5) studied the same phenomena in albino rats. They found that the plasma phosphatase in albino rats was greatly reduced by fasting. Only the ingestion of the alcohol-soluble fraction of the rat diet increased the plasma phosphatase level. The ingestion of carbohydrates and proteins did not increase the low phosphatase activity

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produced by fasting. The ingestion of saturated fatty acids and saturated dicarboxylic acids gave the same results as the ingestion of carbohydrates and proteins. An increase in the low plasma phosphatase activity produced by fasting resulted only from the ingestion of certain unsaturated fatty acids wherein a certain molecular size, a double-bond in the carbon chain, and the presence of free carboxylic groups was essential. Cephalin ingestion after fasting produced a marked increase in plasma phosphatase activity while under the same conditions lecithin was found to be entirely without action. Gould(6) also found that prolonged feeding of fats to rats resulted in an increase in serum phosphatase to extremely high levels. He suggested that the increase in serum phosphatase after fat-feeding and the decrease after fasting were due to a quantitative alteration of the enzyme rather than to the action of inhibiting or accelerating agents.

From these reports it can be seen that both in dogs as well as in rats there is marked fall of plasma phosphatase activity on fasting. While the active food factor responsible for bringing up the phosphatase activity in fasted dogs is carbohydrate, fat seems to be the important factor in rats. So far no data are available regarding the effect of diet on the serum phosphatase in men.

Methods. Albino rats were used for the first part of the experiment. They were normally fed Purina laboratory chow. The normal levels of alkaline phosphatase were estimated on samples of blood taken in the morning from animals kept on the usual diet. About 0.75 ml of blood was drawn from the tail each time and 0.25 ml of serum used in each estimation. The Binkley, Shank, and Hoagland(7) modification of the King-Armstrong method was used for the estimation of alkaline phosphatase. Twelve rats averaging 400 g in body weight were numbered and their normal serum phosphatases were estimated. They were then fasted for 24 hours and tested again for enzyme activity. Another group of 6 rats were adrenalectomized and their phosphatase levels were estimated 2 weeks after adrenalectomy both before and after fasting. Three groups of 4 rats each were selected for the feeding of carbohydrates, proteins, and fats separately. Five to 7 ml of 25% glucose solution were administered by stomach tube to one group, the same quantities of egg-albumin solution were administered to the second group, and about 5 ml of ether-extract of egg yolk were given to the third group. The serum phosphatase was estimated in each case under normal fed condition, after fasting for 24 hours, and 2 hours after feeding special diets. The same types of experiments were conducted on men. Patients from Grady Hospital who had no liver or bone diseases and who were not seriously ill were selected for the study. Three to 4 ml of blood were drawn each time and 1 ml of serum was used for each estimation. For the determination of normal levels of phosphatase, blood samples were drawn about 2 hours after the normal breakfast from patients kept on the normal diet throughout the previous day. Nine patients were tested for serum phosphatase activity under normal fed conditions. The same patients were tested again after about 20 hours of fasting. (They were not allowed to eat anything from 12 noon to 8 a.m. on the next day). Special feeding experiments were done on smaller groups of subjects. For a carbohydrate diet, a breakfast consisting of breadtoast, jam, and orange juice was allowed to 4 men who had been previously fasted for 20 hours. Blood specimens were taken before feeding and 2 hours after feeding. For a protein diet, the whites of hard-boiled eggs and skimmed milk were given. It was rather difficult to administer pure fat to patients. Hence, a diet with scrambled yolk of eggs and plenty of butter was given to the third group of subjects. Three individuals were tested with protein feeding and 6 others with fat feeding. Out of the 6 subjects fed the fat-rich breakfast, 3 were tested 2 hours after feeding, and the rest 4 hours after feeding.

Results. Table I shows that the normal alkaline phosphatase level in the younger age-group of rats is higher than that in the older ones. But in both these groups there is significant lowering of serum phosphatase on fasting for 24 hours. Adrenalectomy does not influence the effect of fasting on serum phos-

TABLE I. Serum Alkaline Phosphatase in Fed and Fasted Normal and Adrenalectomized Rats.

No. of rats	Avg wt	Serum alkaline phosphatase (King Armstrong units) Unfasted	Fasted	Difference
12	400 g (normal)	23.0±5*	11.7±2.4	11.4±2.8
6	175 g (adrex)	26.0±5.1	12.3±1.4	13.7±2.2

* Stand. dev.

TABLE II. Serum Alkaline Phosphatase in Normal Rats* under Various Conditions of Feeding.

Serum alkaline phosphatase (King Armstrong units) Unfasted	Fasted	2 hr after glucose ingestion
27.6 ± 5.6†	13.9 ± 3.3	13.2 ± 2.4
25.3 ± 4.6	12.4 ± 2.5	Y_1 12.7 ± 2.8
25.3 ± 4.6	12.4 ± 2.5	Y_2 20.1 ± 3.7

4 rats in each series.
Y_1 = 2 hr after protein ingestion.
Y_2 = " " " fat " .
* Avg wt 400 g.
† Stand. dev.

TABLE III. Serum Alkaline Phosphatase in Fed and Fasted Human Beings.

	Serum alkaline phosphatase (King Armstrong units) Unfasted	Fasted	Difference
	4.9	3.3	1.6
	4.1	1.9	2.2
	4.6	2.8	1.6
	5.2	3.0	2.2
	5.4	3.1	2.3
	8.4	4.0	4.4
	9.4	5.4	4.0
	7.5	3.7	3.8
	7.6	3.5	4.1
Avg	6.3 ± 1.9	3.5 ± 1	2.9 ± 1

phatase. Table II shows that the fall in phosphatase activity produced by fasting is not overcome by feeding either carbohydrates or proteins, whereas on feeding of fat the phosphatase activity returns to very near the normal level. Table III shows that the normal serum alkaline phosphatase in humans is not as high as in rats. However, there is a definite lowering of the activity after fasting for 20 hours. Table IV shows that the low level of alkaline phosphatase in man due to fasting is not overcome by the ingestion of carbohydrate food or protein, but that 2 to 4 hours after feeding fat a definite increase occurs.

Discussion. Tuba and Robinson(8) have recently demonstrated that the alkaline phosphatase of rat serum is composed of a fraction which is unaffected by fasting and a second larger portion, which responds relatively quickly to fasting and to ingestion of fat. They believe that this variable or adaptive fraction is largely derived from the intestine, where it is essential in the absorption of fat. Since the alkaline phosphatase response to alteration in diet is the same in the rat and human being, it would seem likely that this determination measures the activity of several enzymes arising from different sources. Although the activity of these enzymes in the plasma is frequently associated with metabolic changes in bone(9), it is apparent that the phosphatase activity of plasma is associated with enzyme activity of other organs as well. As our understanding increases of the normal relationship of serum phosphatases to metabolic processes, the alteration of these enzymes in disease will have new meaning.

Summary. 1. Serum alkaline phosphatase in albino rats was found to be lowered on fasting and was increased by the ingestion of

TABLE IV. Serum Alkaline Phosphatase in Fasted Human Beings and on Subsequent Feeding with Carbohydrate, Protein or Fat.

	Fasted	2 hr after feeding carbohydrates
	3.1	3.1
	4.2	4.0
	4.3	3.9
	4.1	3.8
Avg	3.9 ± .35	3.7 ± .3
	Fasted	2 hr after feeding proteins
	4.0	3.7
	3.0	2.9
	3.6	3.1
Avg	3.5 ± .5	3.2 ± .6
	Fasted	2 hr after fat diet
	4.2	6.6
	3.1	5.2
	4.1	6.4
Avg	3.8 ± .8	6.1 ± .7
	Fasted	4 hr after fat diet
	2.9	5.4
	4.0	7.4
	3.3	6.2
Avg	3.4 ± .55	6.2 ± .9

fat. Carbohydrates and proteins had no effect. 2. The same phenomenon was found in human beings and factors influencing alkaline phosphatase serum levels are discussed.

1. Kay, H. D., *J. Biol. Chem.*, 1930, v89, 269.
2. Gould, B. S., and Schwachman, H., *Am. J. Physiol.*, 1942, v135, 185.
3. Armstrong, A. R., and Banting, F. G., *Can. Med. Assn. J.*, 1935, v33, 243.
4. Bodansky, A., *J. Biol. Chem.*, 1934, v104, 473.
5. Weil, L., and Russell, M. A., *ibid.*, 1940, v136, 9.
6. Gould, B. S., *Arch. Biochem.*, 1944, v4, 175.
7. Binkley, Francis, Shank, R. E., and Hoagland, Charles L., *J. Biol. Chem.*, 1944, v156, 253.
8. Tuba, Jules, and Robinson, Margaret I., *ibid*, 1953, v203, 947.
9. Gutman, Alex B., and Jones, Barbara, PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 572.

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Elaboration of Steroid Hormones by Surviving Adrenal Tissue Slices Obtained from Thermally Stressed Dogs.* (20736)

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In a previous communication(1), a rapid method was described for estimating the secretory activity of surviving adrenal cortical tissue slices. Essentially, the method consisted of incubating dog adrenal cortical tissue in the animal's own serum with added antibiotics in a Warburg apparatus for 24 hours. Following incubation, the hormones were extracted from the tissue and medium, purified by florosil column and paper chromatography methods and quantitatively estimated. The amount of the individual hormones elaborated by surviving adrenal cortical tissue from normal dogs was found to be fairly constant when the value was expressed as micrograms of hormone per milligram of adrenal cortical nitrogen.

Methods. Healthy adult male dogs were anaesthetized with nembutal, shaved, and 30% of the body surface burned by multiple exposure to a bank of 43 750-watt reflector photolamps for 0.8 second(2). This exposure was sufficient to cause a third or deep second degree burn. The dogs received no supportive therapy and were allowed food and water *ad libitum*. Four dogs were sacrificed 24 hours after burning and 4 others 72 hours after burning. The adrenal glands were incubated as described(1) except that two incubation vessels were used, one with and one without corticotropin.† Five dogs were given large doses of corticotropin‡ over a period of 24 hours and sacrificed immediately. Two dogs received the corticotropin intramuscularly in divided doses at 8-hour intervals, and three by continuous intravenous infusion of corticotropin dissolved in 5% glucose in water. Slices of adrenal tissue were similarly incubated.

Results. As may be seen in the Table, there was a marked increase in the amount of hormone produced by the adrenal slices obtained from dogs sacrificed 24 hours after burning compared with the values obtained by incubating adrenal slices from normal dogs. The dogs which received corticotropin for 24 hours prior to sacrifice also showed increased elaboration of steroid hormones, but the increase was less marked than in the burned animals particularly with respect to 17-hydroxycorticosterone (Compound F). There was no significant difference observed between the dogs which received the corticotropin by I.M. or I.V. injection. The adrenal slices from the dogs which were sacrificed 72 hours after burning showed

* The opinions expressed herein are those of the authors and should not be construed as necessarily representing the opinion of the Naval Service.

† Each vessel contained 10 mg of corticotropin (Armour lot No. 128-105R, potency 1.6 times that of U.S.P. standard) supplied by Dr. Irby Bunding, Armour Laboratories, Chicago.

‡ Approximately 7 U. S. P. units/Kg of ACTHAR®.