previously reported(1). Following ingestion of the radioactive ascorbic acid an appreciable amount of $C^{14}O_2$ is present in the expired air within the first half hour and reaches its peak by the third hour. Thereafter there is a precipitous decline in excretion of $C^{14}O_2$ which levels off by the 10th and 12th hours. The specific activity of $C^{14}O_2$ per mole of CO_2 is above 1% per hour of the ingested radioactivity during the 2- and 3-hour periods. Specific activity is below 1% per hour except for the period from 2 to 4 hours following ingestion (Fig. 2). After 24 hours specific activity falls to 0.1% of the ingested amount of radioactive ascorbic acid.

Conclusion. As previously pointed out in the literature, a certain portion of ascorbic acid administered to the human could not be accounted for before the availability of radioactive compounds(13,14). We have shown that this unknown balance can now be accounted for as carbon dioxide excreted in the exhaled air. The catabolism of ascorbic acid is relatively slow in the human. An appreciable amount of radioactive ascorbic acid must therefore be ingested in order that the catabolized carbon dioxide can be detected in man. It is therefore necessary to ingest quantities in the range of 1 μc of L-ascorbic-1-C^{14} acid per lb of body weight to measure catabolized $C^{14}O_2$ in the expired air with an ionization chamber or a liquid scintillation spectrometer. Important contributions to human nutrition and the nutritional requirements of vit C may be derived from the demonstration of this new human excretory pathway in the catabolism of ascorbic acid.

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Effect of Alterations in Serum Electrolytes on Production of Endogenous Pyrogen.* (29748)

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It is generally believed that fever is mediated by a humoral substance released from leukocytes in response to some form of tissue injury. The pyrogenic material then enters the circulation to exert its influence on the thermoregulatory centers of the brain, thereby initiating the constellation of physiological events which culminate in fever. This fever-promoting substance, termed endogenous pyrogen (EP), has been demonstrated

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in the serum, plasma and tissue fluids of animals made febrile by injection of endotoxin or colloidal materials, by infection with certain influenza viruses, staphylococci, streptococci, and pneumococci as well as during fever associated with tuberculin hypersensitivity(1).

Of many tissues examined, only the polymorphonuclear leukocyte has consistently yielded a pyrogenic material(2). This substance, leukocytic pyrogen (LP), may be biologically similar to the endogenous pyrogens found in serum after a variety of pyrogenic stimuli. Because leukocytes can be readily separated from other components of blood, most biochemical studies dealing with endogenous pyrogens have been performed with the substance derived from leukocytes. LP is readily extractable by incubation of viable cells in saline at 37°C. However, if plasma is substituted for saline, release of LP is markedly retarded unless endotoxin is added to the incubate(3). It has also been observed that granulocytes obtained from the peripheral blood release much less pyrogen when incubated in saline than identical cells obtained from sterile saline-induced peritoneal exudates(4). The reluctance with which leukocytes from blood release their pyrogen can be reversed by addition of the non-cellular fraction of inflammatory exudates to the cells. This observation led Berlin and Wood to postulate the existence of an activator factor in inflammatory exudates which renders the cells more amenable to release of pyrogen(4).

These investigators also have demonstrated that potassium ion may be of great importance in regulating the release of pyrogen from leukocytes *in vitro*(4). They observed that in physiological concentration potassium inhibited release of LP; when the concentration of potassium in the incubating medium was lowered, the cells readily gave up the pyrogen. Inhibition appeared to be more directly related to the concentration of extracellular than intracellular ion and was nearly maximal at 5 meq per liter. Calcium, cesium, rubidium and a few other cations also possessed inhibitory activity qualitatively similar to potassium although much less striking(4). In view of these findings, it seemed of interest to investigate the effect of hypokalemia on the elaboration of endogenous pyrogen in serum following the experimental production of fever.

Materials and methods. All experiments were performed on rabbits. Endogenous pyrogen (EP) was prepared in normal animals and animals with electrolyte imbalance by intravenous injections of 3.3×10^8, 6.6×10^7 and 3.3×10^7 heat-killed *Salmonella typhosa* into groups of 6 normal rabbits. Animals were exsanguinated at the height of the febrile response 120 minutes later. Blood was allowed to clot at room temperature, and serum cleared by centrifugation at 4°C. Sera were cultured, and, if sterile, were pooled and stored at 4°C until tested.

For certain experiments a potassium-deficient diet was obtained (Nutritional Biochemicals Co., Cleveland, Ohio) to which benzoic acid was added as a preservative. Control animals in these experiments received Purina lab chow. Oral intake was *ad lib* and tap water was constantly available to all rabbits.

Acute hypokalemia was produced in rabbits fasted during the previous 12 hours. In these animals 10 ml/kg of a 5% suspension of sodium carboxylic acid cation-exchange resin (Eli Lilly & Co.) dissolved in tap water, was instilled into the colon through a 14 French urinary catheter. Acute, combined hyperkalemia and hypercalcemia was produced by rectal instillation of a 1:1 mixture of 2% KCl and 1% calcium gluconate in tap water. Animals were given 10.0 ml/kg of this mixture exactly as described for the resin enema. Concentrations of calcium were raised by intraperitoneal instillation of 2% calcium gluconate in dosage of 10.0 ml/kg. Each group of animals with an electrolyte imbalance consisted of at least 6 donors.

For determinations of leukocyte counts and electrolyte concentrations, blood was obtained by cardiac puncture and anticoagulated with heparin. Plasma was separated by centrifugation at room temperature 30 to 90 minutes after exsanguination. Leukocyte counts were performed in duplicate on heparinized blood using an ordinary clinical he-

mocytometer. Potassium was determined in duplicate on a Baird Flame photometer and calcium was measured in duplicate by the Clark-Collip modification of the Kramer-Tisdall method(5).

EP was assayed by construction of a dose response curve and calculation of the minimum pyrogenic dose (mpd). The method for the assay and its validity have been described (6).

Results. Three groups of 6 rabbits each were placed on a potassium-deficient diet while 3 control groups of 6 animals each received ordinary lab chow. After 3 weeks of this dietary regimen, the mean concentration of K^+ in plasma among test animals was 2.2 ± .25 (S.D.) meq per liter, well below the normal range of 4.0 ± .8 meq/l; the mean leukocyte count of these animals was 7520 cells per mm^3 (normal: 6000-9000). All animals in each group were then tested for their ability to produce EP following challenge with 3 graded doses of typhoid vaccine. All animals became febrile and were exsanguinated 120 minutes after injection of the vaccine. Sera from each group were pooled and assayed for pyrogenicity. The amount of EP released by normal and hypokalemic rabbits is summarized in Fig. 1. At each of the 3 dosages of typhoid vaccine tested, potassium-deficient animals released much more EP than normals. Following injection of 3.3×10^8 cells, the mpd for normal animals was 3.0 ml, while the serum of hypokalemic animals contained the same amount of EP in 0.8 ml. Likewise, the mpd for normals given 6.6×10^7 cells was 7.2 ml, while it was only 1.8 ml in potassium depleted animals. The same relationship held true at the lowest dosage of typhoid vaccine (mpd of normals 13.8 ml; mpd of hypokalemic animals 5.8 ml).

Rabbits were acutely depleted of serum potassium by means of a carboxylic acid cation-exchange resin enema. Twenty to thirty minutes after instillation of a suspension of this material, 6 animals were injected with approximately 6.6×10^7 killed *S. typhosa*; they were exsanguinated 120 minutes later and their sera assayed for pyrogenicity. Plasma potassium levels were determined prior

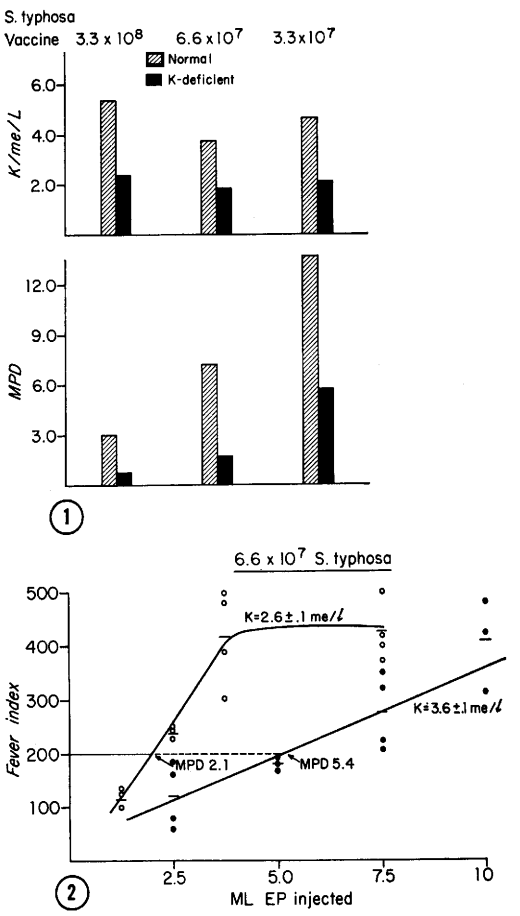

FIG. 1. Comparison of minimal pyrogenic dose and concentration of K^+ in normal and hypokalemic rabbits.

FIG. 2. Pyrogenicity of serum EP from rabbits made acutely potassium deficient.

to the enema, immediately prior to injection of the vaccine and again at time of exsanguination. Although not as marked as in the chronically deficient animals, significant hypokalemia was produced with a fall of the K^+ in plasma from 3.6 ± .12 to 2.6 ± .14 meq per liter. Again serum was found to be more pyrogenic than that obtained from controls (Fig. 2). The mpd of EP from hypokalemic animals was 2.1 ml as compared to 5.4 ml for controls. The enhanced production of EP in these acutely potassium-depleted animals was less marked than in animals which had been on the low potassium diet, perhaps because the degree of hypokalemia was not as marked.

The effect of an acute elevation in plasma potassium on release of EP was studied. This involved considerable difficulty since rabbits subjected to acute increases in plasma K^+ concentration frequently developed ventricular fibrillation and died before the experiment could be completed. The lethal effect of hyperkalemia was overcome to some extent by simultaneous elevation of the serum calcium. Acute, combined hyperkalemia and hypercalcemia was produced in a group of 6 donors by rectal instillation of a solution containing 2% potassium chloride and 1% calcium gluconate. The mean K^+ level of this group of animals was raised from 4.4 $\pm$ .3 to 8.8 $\pm$ 1.2 meq per liter while the plasma calcium concentration increased from 14.0 $\pm$ 0.6 to 16.3 $\pm$ 0.4 mg%. When challenged with 6.6×10^7 *S. typhosa* there was no significant difference between the amount of pyrogen liberated by these animals and the control group, and the mpd was approximately the same for both, 5.7 ml for normokalemic and 6.3 for hyperkalemic animals.

Although it was not possible to produce hyperkalemia alone in these animals, elevation in calcium alone was achieved by intraperitoneal instillation of calcium gluconate resulting in a rise of serum calcium from 15.2 $\pm$ 0.4 to 16.7 $\pm$ 0.2 mg%. EP from these donors had approximately the same degree of pyrogenicity as from controls, providing indirect evidence that the production of hypercalcemia did not alter the effect of hyperkalemic serum on the release of EP.

Discussion. These experiments demonstrate that hypokalemia in rabbits is associated with enhanced production of endogenous pyrogen following a pyrogenic stimulus. In every instance in which animals were given the same dose of endotoxin, K^+ deficient donors released more EP than normals. In a limited sense, these data represent an extension of the observations of Berlin and Wood to the intact animal. It would be naive to suppose that the unicellular system employed by Berlin and Wood to demonstrate the effect of K^+ on release of cellular pyrogen has an exact parallel in the intact organism. It is conceivable that simultaneous alterations in sodium, calcium, magnesium and other cations as well as other changes which might have been affected by the changes in K^+ concentration, played some role in determining the production of serum EP. This does not gainsay the fact, however, that the concentrations of both intracellular and extracellular electrolytes may exert a profound effect on the release of EP, a factor ignored in previous studies. Hemoconcentration did not play a role in potentiating the pyrogen since packed cell volume did not change in animals subjected to chronic K^+ depletion.

Furthermore, these results provide another indirect link in the chain of evidence that leukocytic pyrogen obtained from sterile inflammatory exudates is biologically identical to endogenous pyrogen in the serum of febrile animals. While significant amounts of pyrogen are released from leukocytes in inflammatory exudates in the presence of physiological concentration of K^+(7), the same held true for the normokalemic animal given endotoxin in the present study. Whether EP or LP are, in fact, identical must await their biochemical characterization.

High concentrations of K^+ did not depress the release of EP. These results were not unexpected since it had been demonstrated *in vitro* that maximum inhibition of EP release occurred at physiological concentrations and that further inhibition could not be achieved by increasing the concentration of K^+ to supernormal levels(4).

The studies of Berlin and Wood *in vitro,* as well as the present studies in intact animals, point up the importance of the extracellular environment in the phenomenon of cell damage. It is conceivable that other anions and cations present in body fluids may play a vital role in protecting the cell from injury, or conversely, in rendering it more susceptible, and thus to some extent determine the release of endogenous pyrogen and the magnitude and duration of fever.

Summary. Rabbits acutely depleted of serum potassium or made chronically potassium deficient released more endogenous pyrogen following a constant pyrogenic stimulus than normokalemic controls. Increase of serum potassium to levels of 8.8 meq/l did not alter the animals' ability to release endogenous

pyrogen, and production of hypercalcemia also had no effect. These observations confirm the inhibitory effect of potassium on release of pyrogen from leukocytes *in vitro*.

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3,5 Diiodothyroacetic Acid Mobilization of Rat Liver Lipid and Relative Toxicity of Oral Triac and Triprop. (29749)

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In earlier studies, a series of thyroxine-like compounds was tested for their relative effects on tissue cholesterol and lipid concentrations, growth and oxygen consumption rates in hypercholesterolemic rats(1,2). The purpose of these investigations was to find a thyroxine analogue(s) which would lower tissue cholesterol concentrations without significantly affecting other biological parameters. The animals were fed a 29% lard, 19% casein, 1% cholesterol purified diet, and thyroxine analogues were either fed or injected in an effort to prevent or reverse the condition of hypercholesterolemia. Of the compounds tested, 3,5 diiodothyroacetic acid (diac) appeared to show the greatest preferential effect on cholesterol and lipid concentrations without significantly affecting oxygen consumption and growth rates. Diac was more effective when fed in the diet than when injected intraperitoneally at a comparable dose level, and optimum dietary concentration of diac was found to be 5 mg%.

Additional studies have been made of the effects of diac on the mobilization of liver and plasma lipid concentrations. The total decrease in liver weight produced by diac feeding has been accounted for, and the conditions for optimum lipid mobilization have been determined. Two other thyroxine derivatives, 3,5,3′ triiodothyroacetic acid (triac) and 3,5,3′ triiodothyropropionic acid (triprop) were found to be more toxic than T_4 when fed in the diet although they have only 20 and 10% respectively the calorigenic activity of T_4 when injected subcutaneously.

Methods. It had been noted in previous experiments that the livers of animals receiving a high fat-cholesterol-cholic acid diet were enlarged, and that the liver size was reduced to control levels when diac was administered. It was proposed to determine the nature of the substances mobilized, and to assess the effects of the level of dietary fat and protein on the diac effect. A large group of weanling male rats was started on the high fat-cholesterol-cholic acid diet which was composed fundamentally of 19% casein, 26% lard, 1% cholesterol, 0.5% cholic acid and sucrose, vitamins and salts to make 100%. The actual composition of the diet has been described(2). The animals were housed by pairs in wire mesh bottom cages (8 animals per group) in a room maintained at a constant temperature of 25°C. Water and food were supplied *ad libitum*. At the end of 3 weeks, 8 animals were sacrificed and liver weights, plasma cholesterol, liver cholesterol, total liver lipid, liver total reducing sugar and liver water determinations were performed.

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