normal; the surviving cells are reduced in size(3). It is entirely conceivable that the residual fibers are the same type as those found in control animals except that the antiserum treatment has in some way reduced the ability of the nerve to develop an electrical potential. If this were the case the failure of electrical stimulation of these nerves to produce vasoconstriction could be explained on the basis that a threshold potential necessary for release of norepinephrine (from the nerve terminals) was not reached.

The present results do not allow for a conclusion as to which factor is responsible for the type of activity recorded from the sympathetic fibers of rats treated with the anti-nerve growth factor. It may be that neither of these factors ultimately contributes to the finding that the sympathetic nerves in the immunized animals fail to exhibit adrenergic function. The significant loss of norepinephrine content in structures innervated by the sympathetic nervous system following immunological sympathectomy(5) must also be taken into consideration.

Summary. Electrical activity was recorded from the lumbar sympathetic chains of control rats and rats treated with an antiserum developed to the sympathetic nerve growth factor derived from mouse salivary glands. The magnitude of the potentials recorded from the nerves of the immunized animals was considerably smaller than the magnitude of discharge seen in control animals. These observations provide further evidence for the effectiveness with which the anti-nerve growth factor damages the sympathetic nervous system. Several possible explanations for the type of activity recorded from the nerves of immunized animals were considered.

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Species-Specificity of Endogenous Pyrogen in Serum.* (28733)

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Intravenous administration of bacterial endotoxin into rabbits and dogs is followed by the appearance in the serum of a secondary fever-producing substance, endogenous pyrogen (EP), which differs in its biological properties from the endotoxin originally administered(1). EP is presumably derived from granulocytes injured by the endotoxin. These cells are the only tissues consistently pyrogenic when injected into animals of the homologous species(2). Furthermore, animals made leukopenic with nitrogen mustard produce less endogenous pyrogen(3) and have less fever(4) than controls.

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Endogenous pyrogen (EP) in the serum of febrile animals and pyrogen derived from polymorphonuclear leukocytes (LP) have been presumed to be biologically equivalent. In ordinary dosage both produce monophasic febrile responses occurring after a brief latent period, both are active in animals made tolerant to bacterial endotoxin and both are inactivated by heating at 90°C for 30 minutes(5). However, there is at least one difference in the biological properties of these two pyrogens. Whereas LP is pyrogenic only in species from which it is derived(6), preliminary experiments have demonstrated that serum EP from rabbits given typhoid vaccine is pyrogenic in dogs and vice versa(7).

The lack of species specificity of EP was independent of the stimulus employed to produce the pyrogen and EP obtained following injection of Newcastle disease virus was also pyrogenic in heterologous species(7). In the studies to be described a more sensitive assay was employed to determine pyrogenicity of EP in rabbits and dogs and the fever-producing effect of human serum obtained from febrile patients was assessed in rabbits.

Materials and methods. Male 1.6-4.0 kg white rabbits of mixed breed and 8-20 kg mongrel dogs were used as donors of pyrogenic serum. Donor animals were injected intravenously with heat-killed *Salmonella typhosa* vaccine containing 10^8 killed bacterial cells per ml. Each animal was given vaccine in dosage of 1.0 ml/kg body weight. Animals were exsanguinated by cardiac puncture at the height of fever, 120 minutes after administration of vaccine. Blood was permitted to clot at room temperature, stored overnight at 4°C and serum cleared by centrifugation at 2000 RPM for 2 hours. Serum was cultured in thioglycollate broth at 37°C and all samples showing bacterial contamination were discarded. Sterile sera from donors of each species were pooled and stored at 4°C until used.

50-100 ml of blood were obtained by vein puncture from 15 patients during fever greater than 102°F; a second sample was taken from each patient 72 hours after defervescence. The causes of fever varied and clinical diagnoses included pneumococcal pneumonia, tuberculosis, barbiturate intoxication, peptic ulcer, mumps, diabetes mellitus, Cushing's syndrome, renal calculi, cerebral infarction, pyelonephritis, disseminated carcinomatosis, acute pancreatitis, intestinal obstruction, emphysema, bronchitis, Laennec's cirrhosis, influenza and viral pneumonia. Patients with bacteremia and endotoxin shock were excluded. All temperatures in patients were taken rectally with ordinary clinical thermometers.

Pyrogenicity of sera was assayed in rabbits and dogs. The former were placed in metal stalls with open backs and tops and were restrained with loose fitting collars. Temperatures in rabbits were recorded with an indwelling rectal thermister (Telethermometer, Yellow Springs Instrument Co.). Acclimatization was permitted to take place for 2 hours prior to injection of sera and animals with baseline temperature fluctuations greater than 0.2°C were not used. Dogs were kept in cages unrestrained and rectal temperatures taken with clinical thermometers.

To prevent sensitization to serum from donors of heterologous species, test injections involving foreign serum were given on successive days and no recipient animal received more than 2 injections of foreign serum.

Assay of endogenous pyrogen was based upon the injection of graded volumes of serum into normal recipient animals. By this means dose-response curves could be constructed. Temperatures were recorded at 15-minute intervals for 3 hours. Fever curves were plotted on standard graph paper with 10 cm on the vertical axis representing 1°C of fever and 5 cm on the horizontal axis corresponding to 1 hour. Only fever curves conforming to the usual criteria for fever caused by EP, *i.e.*, monophasic fever with a short latent period, were used. This included the majority of pyrogen assays. Two-hour fever indices were determined by measurement of the area beneath each curve with a planimeter (Keuffel-Esser 423 M-9788).

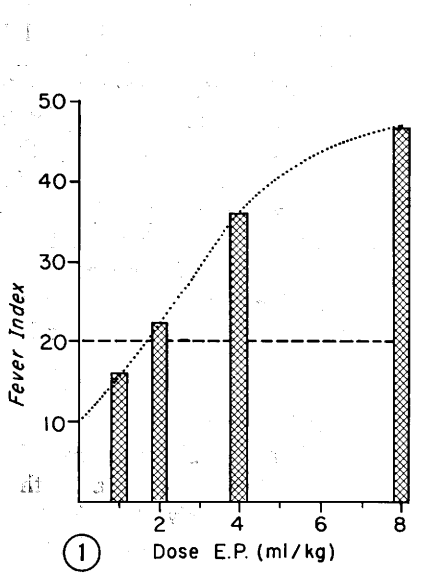
The volume of serum tested was based upon the *minimum pyrogenic dose* (mpd), which was defined as the smallest volume of serum producing a fever index of 20. This point of reference was selected because: (1) it represents a point midway along the steep linear portion of the dose response curve(8, 9); (2) a fever curve with a fever index of 20 had the typical configuration of EP; and (3) a fever index of 20 is only slightly greater than that of a hypothetical fever curve which rises 0.2°C within 15 minutes and remains at that level for 2 hours. After the mpd of a given lot of EP was defined, several multiples of the mpd were tested in order to construct a dose response curve (Fig. 1, 2). Each dose was assayed in at least 5 rabbits and 3 dogs.

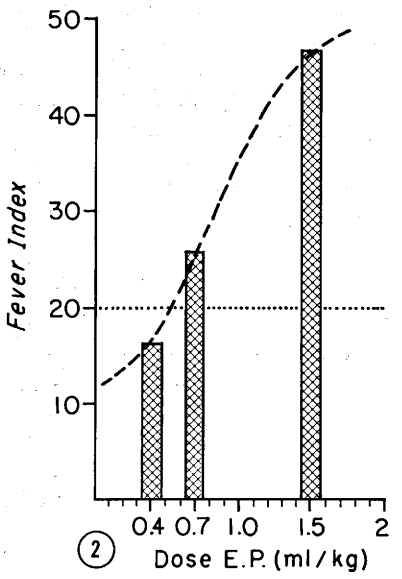
All needles, glassware and instruments were sterilized in hot air ovens at 170°C for

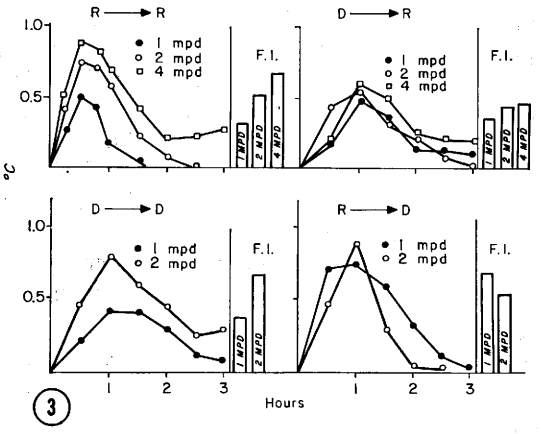
3 hours. All serum and other injectables were cultured in thioglycollate broth and discarded unless sterile.

Results. The dose response curve of rabbit EP in rabbits is depicted in Fig. 1. Approximately 1.8 ml of serum was required to produce a fever index of 20. Larger doses resulted in correspondingly greater elevation in temperature up to a level of 8.0 ml, after which a hyperthermic plateau was reached. A dose response curve of similar configuration was obtained when EP from dogs was tested in animals of the homologous species (Fig. 2). In this instance only 0.6 ml of serum was required to produce fever with a fever index of 20, indicating that the dog is more sensitive to the pyrogenic effect of EP that the rabbit.

When EP from rabbits was administered to dogs, the recipients became febrile and conversely, canine EP was pyrogenic in rabbits (Fig. 3). However, unlike the response to EP from the homologous species, injection of larger volumes of serum into heterologous







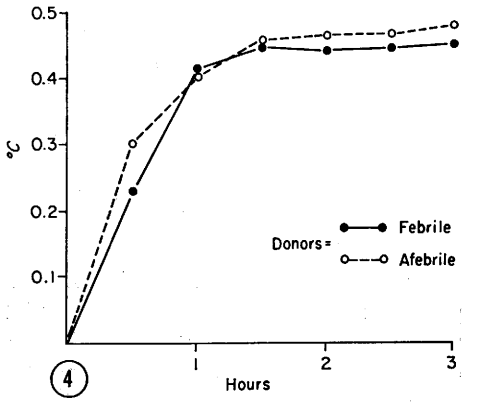


FIG. 1. Dose-response curve of rabbit EP in rabbits.
FIG. 2. Dose-response curve of dog EP in dogs.
FIG. 3. Fever-producing effect of rabbit and canine EP in homologous and heterologous recipients.
FIG. 4. Mean temperature elevations in groups of 3 rabbits given febrile and afebrile sera from each of 15 patients.

recipients failed to result in more fever (Fig. 3). This could not be attributed to the dose of EP since the doses injected were based upon the mpd determined in recipients of the homologous species. Normal dog serum sometimes initiated a mild febrile response in rabbits; the reverse was seldom the case.

Ten ml of blood from 15 patients with fever of diverse etiology was injected into each of 3 normal rabbits and its pyrogenicity was compared with 10.0 ml of serum obtained from each of these patients while they were afebrile. Although there was considerable variation in the pyrogenicity of individual sera, there was no difference in the mean fever-producing capacity of febrile and afebrile sera (Fig. 4). Reduction of the dose to 5.0 ml resulted in some decrease in fever but there was no difference between the fever-producing effect of serum from febrile and afebrile patients. Injection of larger aliquots often resulted in death of the animals.

Discussion. These studies confirm previous observations(7) that serum EP from dogs produces fever in rabbits while rabbit pyrogen is pyrogenic in dogs. Superficially, these results suggest that serum EP is not species-specific. However, the consistent failure of graded doses of EP to produce fever of increasing magnitude in recipients of heterologous species is more compatible with the interpretation that fever associated with heterologous EP is a non-specific response to injection of foreign serum. This is probably an oversimplification in view of the rarity with which normal serum produced fever in recipients of another species. The experiments with human serum also support the idea that rabbits respond to injections of foreign serum with some elevation in temperature, regardless of fever in the donor.

An interesting result derived from these experiments is that canine EP appeared to be more pyrogenic than that obtained from rabbits challenged with the same dose of endotoxin. This may reflect an increase in the amount or potency of pyrogen generated by canine leukocytes or may merely mean that the dog is more sensitive to EP than the rabbit. Since rabbits responded to canine EP with fever of similar magnitude as dogs, the first explanation seems more plausible.

These results emphasize that pyrogen assays must be interpreted in the light of the potency and variability of EP from different species. In addition, performance of these assays in recipients of a different species is likely to be affected by the non-specific pyrogenic effect of foreign serum and, for that reason, should probably be avoided.

Although leukocytic pyrogen did not produce fever in animals of heterologous species, the results of the present study should not be interpreted to mean that endogenous pyrogen in serum and leukocytic pyrogen are not biologically equivalent, or that fever in man is not mediated by EP. With respect to the first point, the data point out only that pyrogen assays in heterologous species involve mechanisms which are poorly understood, particularly when the substance tested contains a large quantity of protein foreign to the recipient. The precise answer to the identity of EP and LP must await their biochemical identification. Finally, it seems unlikely that none of the information incriminating EP in the pathogenesis of experimental fever is applicable to human disease. It is more probable that a method more sensitive than the passive transfer technique will reveal the presence of EP in human fever.

Summary. Endogenous serum pyrogen from rabbits produced fever in dogs and vice versa. Pyrogenicity of EP in the homologous species was dose related, but this was not the case in the heterologous species. Human serum obtained from patients both during fever and after defervescence produced fever in rabbits. These results suggest that pyrogenicity of EP in animals of different species is probably a non-specific phenomenon.

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Changes in Brain Glycogen Concentration in Rats During High Altitude (12,470 ft) Exposure* (28734)

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Although the brain is more susceptible than other tissues to the deleterious effects of hypoxia and hypocapnia at moderate and high altitudes(1), few studies have investigated brain metabolism during altitude acclimatization. On the other hand, the effects on brain metabolism of acute, severe anoxia or asphyxia, *in vivo* and *in vitro*, have been frequently studied(2-12). In general, severe conditions of anoxia decreased the substrates for energy metabolism and increased metabolic end-products in brain. For example, adenosinetriphosphate, phosphocreatine, glycogen or glucose were depleted and lactic acid and inorganic phosphates were increased(2-11, see 11 for additional references). In contrast, a 24-hour fast at reduced atmospheric pressures (280 mm Hg) resulted in a 77% increase in brain glycogen in rats and guinea pigs, whereas fasting alone produced a 6% decrease(12). Also, rats acclimatized to simulated altitudes of 11,000, 18,000 and 22,000 ft for 1-3 months did not show conclusive changes in any of the preceding brain constituents, including brain glycogen(2).

The preceding studies suggest that during altitude exposure brain concentration of metabolic substrates, such as glycogen, would depend on both the severity and duration of the hypoxia. However, conditions during asphyxia and in a decompression chamber do not exactly duplicate those at high altitude. For example, during asphyxia, hypoxia is associated with hypercapnia, whereas at high altitude it is accompanied by hyperventilation-induced hypocapnia(1) and the level of carbon dioxide itself may influence brain metabolism(11). Also, in a decompression chamber, animals usually must be returned to sea level pressures in order to collect tissues for analysis(4).

In the present study, brain glycogen and plasma glucose levels were determined in rats after 3, 8, 30 and 60 days at 12,470 ft elevation and compared with values for sea level controls. Glycogen was measured because it is an important metabolic constituent of brain and because previous work from this laboratory has shown that recovery time after convulsions was prolonged at the same altitude (13), an observation which could be explained by a defect in brain carbohydrate metabolism. The latter appeared probable, because Timiras *et al*(14) have noted that glycogen concentrations in skeletal muscle, heart and liver of the rat are markedly influenced by sojourn at the same elevation.

Methods. The investigations were conducted on the Berkeley campus at an altitude of 250 ft (755 mm Hg) and at the Barcroft Laboratory of the White Mountain Research Station at an elevation of 12,470 ft (480 mm Hg).

Adult male Long-Evans rats with an initial body weight of about 250 g, born at sea level, were used. Conditions of nutrition (pellet diet for rats and water *ad libitum*), caging, temperature, and illumination were comparable in the animal rooms at sea level and at altitude. Animals were taken to the Barcroft Laboratory and sacrificed after 3, 8, 30 and 60 days at altitude. Sea level controls were sacrificed at ages equivalent to those of 8-day

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