Summary. The localization of soluble and insoluble collagen fractions during the development of normal chick embryo tendon and granulation tissue in injured adult chick tendon was studied by the immunohistochemical technique. Antisoluble collagen antibody reacted only with the cytoplasm of young fibroblasts and with extracellular young collagen fibers. Reticulin fibers which precede the collagen and interfibrillar mucopolysaccharides did not react nor did other tissue structures. Antiinsoluble collagen antibody did not react with fibroblasts and reticulin fibers but did react with collagen bundles.

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Febrile Responses to the Intracisternal Injection of Endogenous (Leucocytic) Pyrogen in the Rabbit.* (30264)

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Since the demonstration that fever following intravenous injection of a variety of bacterial agents and viruses is accompanied by the release of an endogenous pyrogen (EP) from host tissues, primarily leucocytes(2,9), attention has been directed to the site of action of this pyrogen and its role in the pathogenesis of fever(1). A current hypothesis suggests that EP produces fever by a direct action of the central nervous system (CNS) thermoregulatory centers(1). This hypothesis is supported indirectly by observations which contrast the effects of intracarotid and intravenous routes of administration(8), but it has not been tested by direct application of EP to the central nervous system(3). In this study endogenous (leucocytic) pyrogen prepared from rabbit leucocytes *in vitro* was applied directly to the CNS by intracisternal injection and the resulting fevers compared to those resulting from the same dose administered intravenously in the same rabbits.

Methods. Female white rabbits (2.6-4.0 kg) served as leucocyte donors and pyrogen recipients. Endogenous (leucocytic) pyrogen (LP) was prepared in a standard fashion(4) from polymorphonuclear leucocytes obtained from sterile saline-induced peritoneal exudates. Two stock solutions of LP were prepared from 2 different peritoneal exudates in such a way that each ml of LP solution contained the pyrogen from approximately 80 $\times$ 10^6 leucocytes. The standard dose was 0.1 ml of the stock solution. This was given undiluted in the intracisternal (i.c.) injections. and diluted to 1.0 ml with pyrogen-free 0.9% saline (Abbott) for intravenous (i.v.) injections.

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TABLE I. Contrasts Between IC and IV Injection.

Rabbit	Maximum T_r (°C) IC	IV	d	Fever index 300 min (cm²) IC	IV	d
1	1.9	.3	1.6	91	4	87
2	2.3	.4	1.9	119	5	114
3	2.3	.4	1.9	123	5	118
4	2.2	.0	2.2	106	0	106
5	2.0	.2	1.8	87	7	80

Doses in all injections 0.1 ml.

Recipient rabbits were first accustomed to the ventilated retaining boxes. Before a test injection, a catheter with thermistor (Yellowsprings Instrument Co., Model 401) was inserted 7 cm into the rectum; a thermistor (YSI, Model 402) was taped to the center of one ear; and a pneumograph (Marey-Manning) adjusted around the thorax. Temperatures were recorded from 2 resistance bridges (YSI Telethermometers, Models 43 and 44) with appropriate scales. Respiratory rate was recorded *via* a tambour and ink-writing kymograph (Phipps-Bird).

Each animal was observed for one hour to ensure stability of rectal temperature (T_r), ear temperature (T_e) and respiratory rate. T_r was read to 0.05°C and T_e to 0.2°C. All experiments were performed in a temperature controlled room at 25°C ± 1°C. When T_r had stabilized to ± 0.1°C for one hour, an injection was performed. Intravenous injections were made in the marginal vein of the ear not being used for T_e measurement. Intracisternal injections followed intramuscular anaesthesia (procaine, 2%) and were by acute posterior puncture between the first cervical vertebra and the occipital bone. Intracisternal injection was always confirmed by prior withdrawal of 0.1-0.2 ml of CSF. At least 2 days elapsed between injections, the i.v. injection always preceding the i.c. injection. The i.c. dose was always injected slowly to reduce the possibility of CNS tissue damage from a jet effect. Measurements were made every 4 minutes during the pre-injection period, every minute after injection for the first 30 minutes, and every 5 minutes thereafter to termination at 5 to 8 hours.

Results. Table I presents the maximum increase in T_r from the baseline T_r and the fever index for 300 minutes (area under the T_r curve when 1.0 cm equals 2 degrees on the ordinate and 20 minutes on the abscissa). These values are all for the same dose and stock solution of EP and demonstrate a greater response in all cases to i.c. injection. Table II shows the replicability of the response on repeated injections in the same animal of the same EP solution.

Fig. 1 shows graphically the mean physiological data corresponding to the paired i.v. and i.c. injections reported in Table I. The rise in rectal temperature and concomitant changes in ear temperature and respiratory rate resulting from i.c. injection were markedly greater in intensity and duration than the transitory effects of the equivalent i.v. injection. Both routes of administration were characterized by a variable "latent period" of 10 to 20 minutes before the onset of fever indicated by a sustained fall in respiratory rate and ear temperature(7). These effects on the heat loss mechanisms preceded the initial rise in rectal temperature by one to five minutes. No consistent difference in "latency" between the i.c. and i.v. responses was observable at the dose used.

Intramuscular injections of procaine that preceded i.c. injection had no effect on the measured parameters. Two control injections of pyrogen-free saline and 3 control injections of heat-denatured (90°C) LP, all injected intracisternally in the standard volume of 0.1 ml, produced no significant or sustained changes in the physiological measurements. These observations appear to eliminate the possibility of contaminating endotoxins (destroyed at 160°C) or of responses due to mechanical or ionic effects of the i.c. injection.

Discussion. These data show that endoge-

TABLE II. Contrast Between IC and IV Injection.

Rabbit	Maximum T_r (°C) IC	IV	d	Fever index 300 min (cm²) IC	IV	d
1	2.4	.6	1.8	116	9	107
1	1.8	.7	1.1	94	18	76
1	2.2	.4	1.8	108	14	94
1	2.6	.3	2.3	132	5	127
1	2.2	.3	1.9	122	5	117
6	1.9	.0	1.9	80	0	80
6	2.1	.1	2.0	98	1	97

Dose in all injections 0.1 ml.

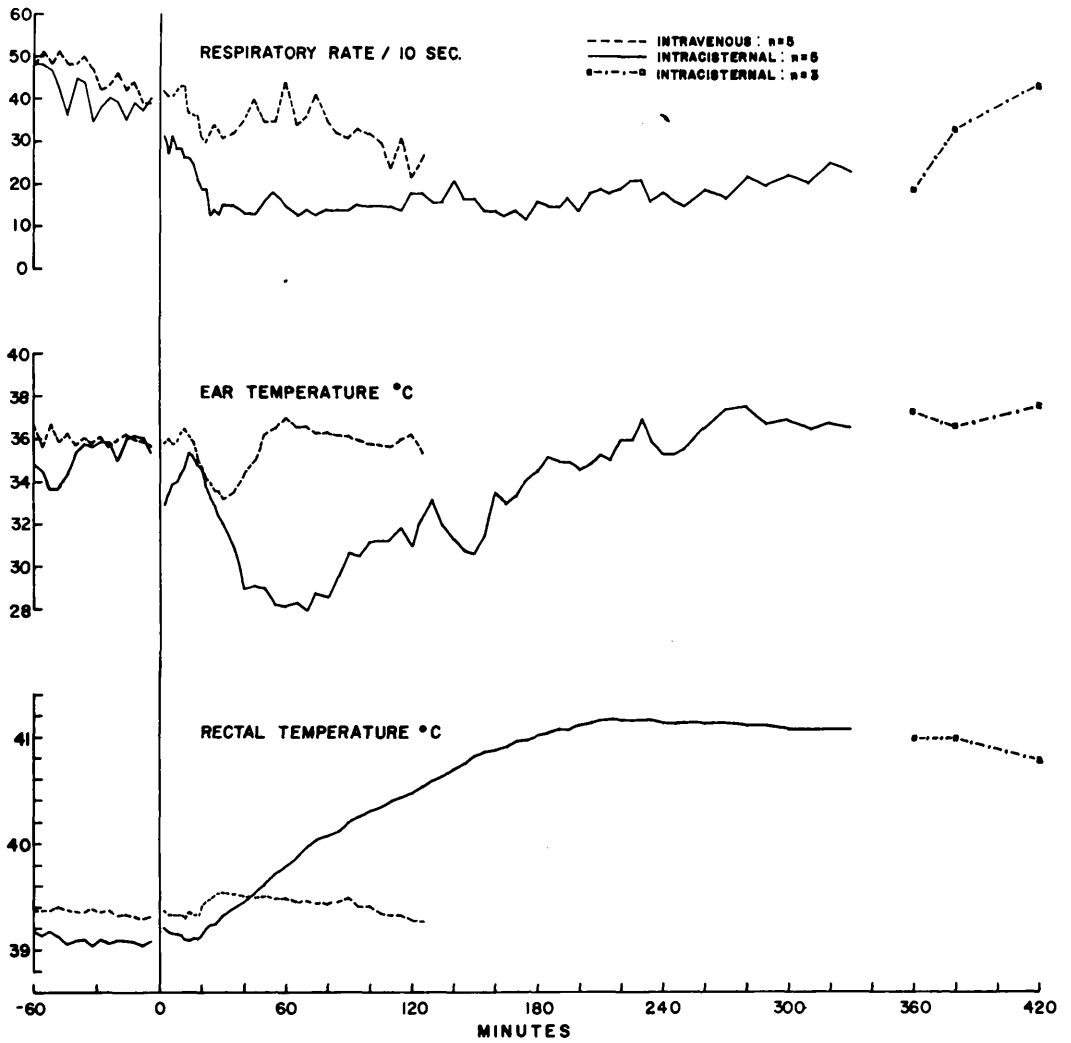

FIG. 1. T_r, T_e and respiratory rate responses of 5 rabbits (Table I) to IC and IV administration of 0.1 ml of EP solution No. 1. Time lapse between end of baseline and end of injection averaged 1-3 minutes for IV injections and 5-10 minutes for IC injections. The disturbance of the IC injection is reflected in slight hyperthermia, mild vasoconstriction and depressed respiratory rate seen in the first 10-15 min after injection. Observations were routinely made only to 330 min on all IC rabbits, but observations from the 300th to 420th min were available on 3 rabbits, and data are presented to show the beginning trend toward baseline of measured parameters.

nous (leucocytic) pyrogen causes intense sustained fever when presented directly to the central nervous system. Identical doses of the pyrogen administered intravenously produced minimal or no fever. The marked difference in response to the two routes of injection is consistent with an hypothesis that endogenous pyrogen has a primary site of action in the CNS, and that this site is exposed to a higher and more persistent concentration of pyrogen following i.c. administration.

The data presented here may be contrasted to that presented in a dose-response study with i.v. administration of LP(4). Ten of 12 of our i.c. injections produced fevers greater than those occurring with 500 times more LP by the intravenous route(4). A maximum febrile response had still not been reached, since doubling our standard dose produced another small increment in T_r

TABLE III. Contrast Between IC Injections at Two Dose Levels.

Rabbit	Maximum T_r (°C) .1 ml	.2 ml	Fever index 300 min (cm²) .1 ml	.2 ml
1	2.2*	2.6	114*	128
2	2.3	2.0	119	104
4	2.2	2.5	106	124
6	2.0†	2.5	89†	130

* Mean of 5 injections—see Table II.
† Mean of 2 injections—see Table II.

(Table III), and we suggest that the log dose fever response curve for LP is dependent upon route of administration. Since similar i.v. doses produced similar fevers in our study (Table I) and the one cited(4), it is unlikely that the higher fevers found with i.c. injection are due to a greater pyrogenicity of our LP.

Although our data clearly indicate that LP has a site of action somewhere in the CNS, they do not locate the site more precisely. The observation that inhibition of heat loss mechanisms precedes the initial rise in rectal temperature is consistent with a direct effect of pyrogens on the medullary-pontine motor centers(5,6), but is also compatible with an indirect effect on these centers secondary to a direct effect on the hypothalamic temperature regulating centers or on postulated pyrogen "receptors"(10) in the CNS.

Summary. Intracisternal injection in rabbits of endogenous (leucocytic) pyrogen prepared from rabbit leucocytes *in vitro* causes a high and sustained elevation of body (rectal) temperature, accompanied by prolonged depression of heat-loss mechanisms (panting and ear blood flow). In paired experiments, the same dose of endogenous pyrogen always caused a much greater increase of body temperature when given intracisternally than when given intravenously. The evidence strongly supports an hypothesis that endogenous pyrogen has a primary site of action in the central nervous system.

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Molecular Size of Rat Urinary Protein. (30265)

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Since the observation that most of the protein of normal rat urine has the electrophoretic mobility of serum globulin(1), many publications have contained the assumption that the normal urinary proteins of the rat have molecular weights equal to or greater than that of serum albumin(2-5), which is 65,000(6,7). The present work demonstrates that a considerable portion of the normal rat urinary protein has a lower molecular weight.

Methods. Urine was collected by bladder massage from adult rats of 3 inbred strains, Buffalo, ACI/N, and Wistar, as well as from Sprague-Dawley non-inbred rats, all of which