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SUPPRESSION OF FEVER IN RABBITS BY A PROTEIN SYNTHESIS INHIBITOR, ANISOMYCIN

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SUMMARY

1. The protein synthesis inhibitor, anisomycin, was given into the cerebral ventricles of rabbits as a priming dose followed by a continuous infusion. Doses of 100, 200 and 300 μg followed by infusions at 100, 200 and 300 μg/hr inhibited the incorporation of [^{14}C] leucine into hypothalamic protein by over 90%.

2. Injection and infusion of anisomycin (300 μg) suppressed the febrile response to leucocyte (endogenous) pyrogen given into the ventricles (I.C.V.) or I.V.

3. Dialysis experiments showed that anisomycin did not combine irreversibly with leucocyte pyrogen.

4. Anisomycin did not interfere with thermoregulation in a cold environment.

5. It is concluded that pyrogenesis may involve a step which is dependent on synthesis of hypothalamic protein with a rapid turnover.

INTRODUCTION

It is generally believed that the fever associated with inflammatory reactions is mediated by endogenous pyrogen, a protein which is released from cells of the reticulo-endothelial system and acts in the preoptic area of the hypothalamus. It has been known for many years that the initial steps in the formation and release of endogenous pyrogen can be inhibited *in vitro* by inhibitors of protein synthesis, such as actinomycin D, cycloheximide and puromycin (for review see Dinarello, 1979). More recently, Siegert, Philipp-Dormston, Radsak & Menzel (1976) have shown that the febrile response to preformed endogenous pyrogen can also be inhibited in the rabbit by the I.V. injection of cycloheximide, and we have confirmed this (Cranston, Dawson, Hellon & Townsend, 1978).

This evidence might suggest a possible role for protein synthesis in the genesis of fever, but Barney, Katovich & Fregly (1979) have pointed out that the systemic treatment of rats with cycloheximide interferes with thermogenesis, rendering the animals unable to maintain a stable core temperature when exposed to a cold environment. Furthermore, Stitt (1980) has shown the same phenomenon in rabbits given cycloheximide I.V., and also demonstrated that cycloheximide does not attenuate the febrile response to endogenous pyrogen in animals in a hot environment, where fever is caused by a reduction in heat loss rather than an increase in heat

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production. He suggested that cycloheximide might have a peripheral action on thermogenesis.

We have therefore examined the effects of a different inhibitor of protein synthesis, anisomycin (Grollman, 1967) on the temperature response of rabbits to endogenous pyrogen prepared *in vitro* from rabbit leucocytes. It has already been shown that anisomycin given systemically can inhibit fever in response to the systemic injection of killed *S. typhi* organisms (Myers, 1980). A short account of these experiments has been communicated to the Physiological Society (Cranston, Hellon, Luff & Townsend, 1980).

METHODS

The effect of anisomycin on protein synthesis in the hypothalamus was measured by a modification of the method of Grahame-Smith (1972). Experiments were performed on rabbits of either sex in a temperature controlled room at 21 ± 1 °C, unless otherwise stated. A week before this, a head plate (Monnier & Gangloff, 1961) was affixed to the skull under general anaesthesia (alphaxalone and alphadolone, Althesin Glaxo) to allow stereotaxic placement of injection cannulae into a lateral ventricle.

Rabbits were restrained in conventional stocks. Their rectal temperatures were measured by indwelling thermocouples inserted 100 mm, and recorded every 10 min on a digital microvoltmeter (Digitec 1268). An injection of 0·1 ml. of rabbit mock c.s.f. (Cameron & Semple, 1968) was made into a lateral cerebral ventricle and 60 minutes allowed to elapse to ensure that there was no pyrogenic contamination of the injection system. The animals were then given an intracerebroventricular (I.C.V.) injection of anisomycin (100, 200 or 300 μg) in a volume of 60 μl. isotonic saline, followed by a continuous infusion of 100, 200 or 300 μg/hr, at a rate of 60 μl./hr. Control animals were given rabbit mock c.s.f. instead of anisomycin. Two hours after the initial treatment, an intraventricular injection of 2·5 μc of L-[U^{14}-C]leucine (342 mc/m-mole, Radiochemical Centre, Amersham) in 50 μl. 2% aqueous ethanol was given. The infusion of anisomycin or c.s.f. was continued for 1 hr, when the animals were killed with pentobarbitone. Hypothalamic tissue extending 3 mm each side of the mid line was rapidly dissected out, and divided into two weighed parts. One part was frozen for later protein estimation by the method of Lowry, Rosebrough, Lewis, Farr & Randall (1951). The other half was homogenised in 10% trichloroacetic acid and extracted, in sequence, with ethanol, ethanol:ether:chloroform (2:2:1), and ether. The final precipitate was dissolved in Soluene-350 (Packard), and 1 ml of this solution added to 10 ml. scintillant (2 l. toluene, 500 ml. Instagel (Packard), 10 g PPO and 125 mg POPOP). This mixture was then counted in a scintillation counter (Intertechnique model SL 30), and corrected to d.p.m./mg protein.

To test the effect of anisomycin on fever, a loading dose of 300 μg was used, with a continuous infusion of 300 μg/hr. The timing of injections of anisomycin and c.s.f. was identical to that in the previous experiments with leucine, and 2 hr after the initial injection, leucocyte pyrogen was given either I.C.V. (15 μl.) or I.V. (2 ml.), temperature being followed for a further 3 hr. In a different experiment, the same timing was followed to determine whether I.C.V. anisomycin affected the febrile response to I.C.V. leucocyte pyrogen in an environment of 27 °C, where the response is mediated principally by a decrease in heat loss.

Non-specific interference with thermogenesis was tested by giving anisomycin or c.s.f., in the same way as indicated above, to animals exposed to a room temperature of 9 °C, and following the rectal temperature for 5 hr.

To test the possibility that anisomycin might have been acting by combining with or inactivating leucocyte pyrogen, the following experiments were performed.

(*a*) 300 μg of anisomycin in 60 μl. saline was incubated with 15 μl. leucocyte pyrogen at 38 °C for 1 hr and injected I.C.V.

(*b*) The same substances were incubated separately under the same conditions and injected I.C.V. simultaneously.

(*c*) 15 μl. leucocyte pyrogen was incubated similarly in 60 μl. saline before I.C.V. injection. Incubation of leucocyte pyrogen alone did not affect its febrile properties. However, when it

was given at the same time as anisomycin, irrespective of whether they had been incubated together or separately, the febrile response was blocked. Hence, there still remained the possibility that anisomycin was combining rapidly with leucocyte pyrogen at or after the moment of injection. To test this, the incubations (*a*) and (*c*) mentioned above were repeated. After incubation, each solution was dialysed against 3 l. saline under sterile conditions for 18 hr at 38 °C to remove free anisomycin.

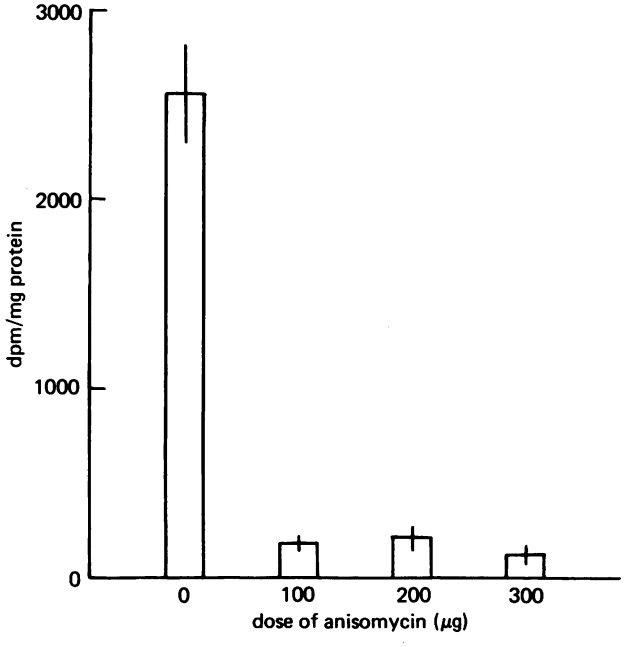

Fig. 1. The effect of three I.C.V. doses of anisomycin on the incorporation of [^{14}C]leucine into hypothalamic protein. Each column shows the mean result from four animals ± S.E. Ordinate shows d.p.m./mg protein; abscissa shows control and three priming doses of anisomycin (100, 200, 300 μg) each of which was followed by a continuous infusion (100, 200, 300 μg/hr) beginning 2 hr before administration of leucine.

Leucocyte pyrogen was prepared by incubating heparinized rabbit blood with typhoid paratyphoid vaccine (TAB, Burroughs Wellcome) in a ratio of four organisms per white cell, for 18 hr at 37 °C. The supernatant was separated by centrifuging at 2000 *g* for 30 min and stored at − 18 °C until used. The same batch of leucocyte pyrogen was used in all the experiments.

In all the procedures, each animal acted as its own control, and the test and control injections were given in a random order. Significance of differences was estimated by paired *t* tests.

RESULTS

Protein synthesis inhibition

The results shown in Fig. 1 indicate that all three doses of anisomycin caused profound reductions in the incorporation of leucine into hypothalamic protein. We elected to use the largest dose which produced a mean reduction of 95 %.

Anisomycin and pyrogen fever

Anisomycin given by the I.C.V. route significantly attenuated the fever following intravenous leucocyte pyrogen, as can be seen in Fig. 2*A*. To confirm that the attenuation was due to a central action, the same dose of anisomycin was given intraven-

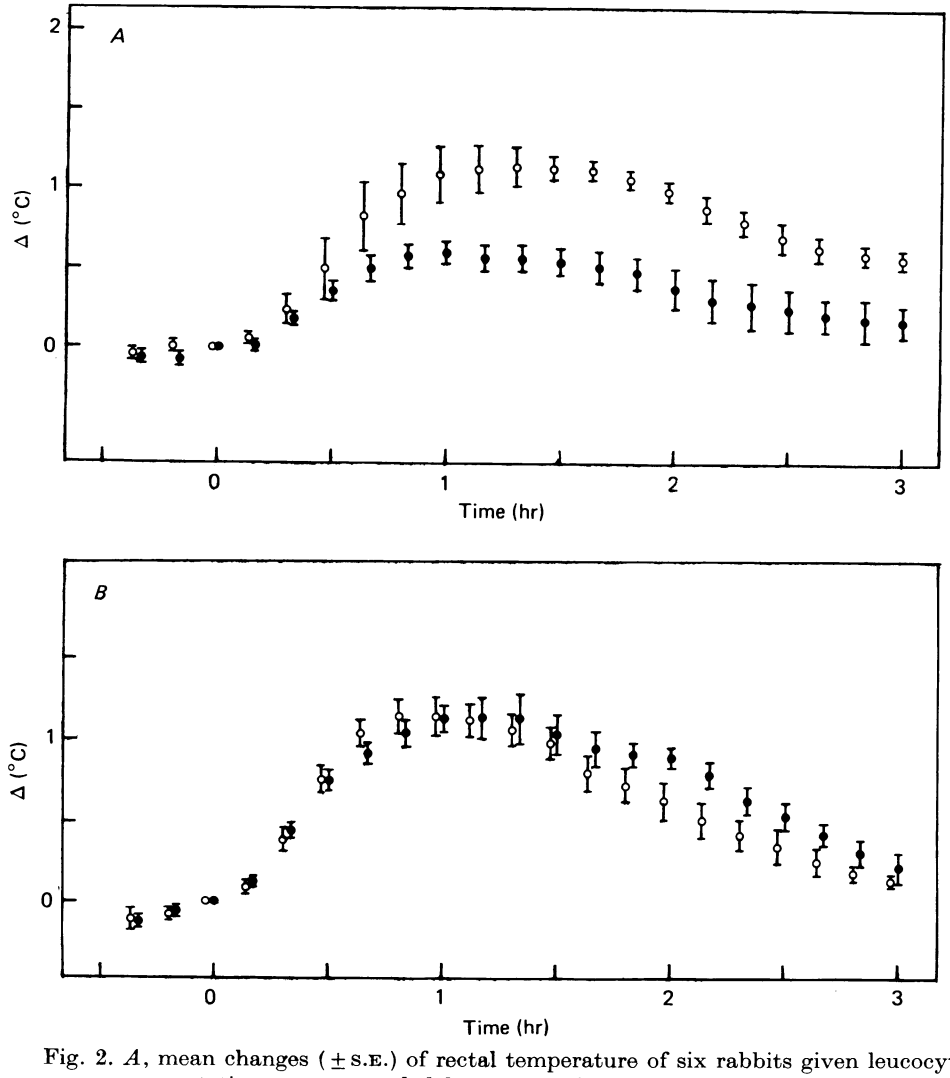

Fig. 2. *A*, mean changes (± S.E.) of rectal temperature of six rabbits given leucocyte pyrogen I.V. at time zero, preceded by I.C.V. anisomycin (300 μg injection and 300 μg/hr infusion) from −2 hr (●) or corresponding volumes of saline (○). *B*, identical experiments to those shown in Fig. 2*A* except that anisomycin was given I.V.

ously and tested against I.V. leucocyte pyrogen. The fever was not different from that found in the control experiments (Fig. 2*B*). A much more complete inhibition of fever was found when both anisomycin and leucocyte pyrogen were given intraventricularly (Fig. 3*A*).

Preliminary incubation of leucocyte pyrogen alone had no effect on its ability to cause fever, as can be seen in Fig. 4*a*. When leucocyte pyrogen and anisomycin were incubated together and then injected, or incubated separately and injected simul-

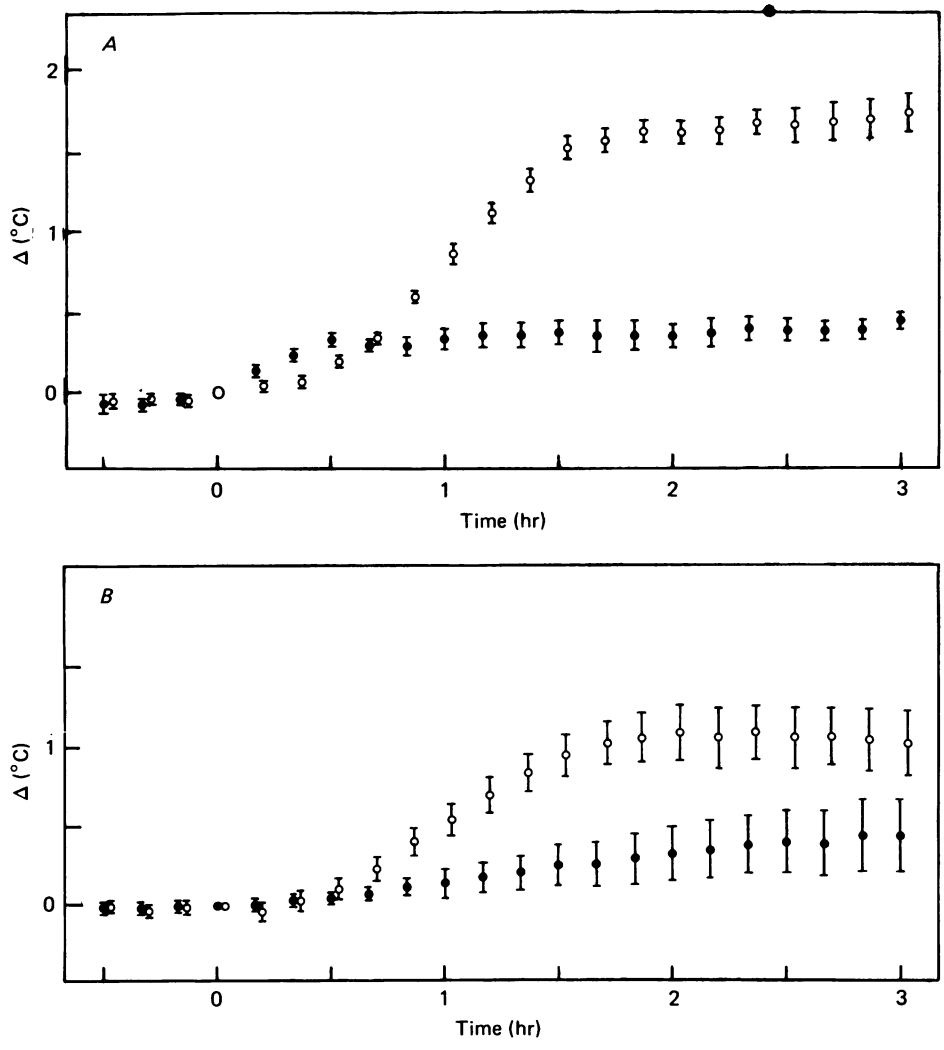

Fig. 3. *A*, mean changes in rectal temperature in six rabbits given I.C.V. anisomycin (300 μg plus 300 μg/hr) at -2 hr (●) or saline (○), and I.C.V. leucocyte pyrogen at time zero. Room temperature was 21 °C. *B*, identical experiments to those shown in Fig. 3*A* except room temperature was 27 °C.

taneously, the response to leucocyte pyrogen was almost eliminated (Fig. 4*A*). The results could be interpreted either as a rapid combination between anisomycin and leucocyte pyrogen, or as a rapid action of anisomycin on some later stage in the fever process. It was to distinguish between these two possibilities that the dialysis tests were performed. When anisomycin and leucocyte pyrogen were incubated together and then dialysed, the subsequent fever response was identical to that due to leucocyte

pyrogen incubated and dialysed without anisomycin. The two responses are shown in Fig. 4*B*.

Anisomycin and thermoregulation

To test whether anisomycin was producing its antipyretic effect by the suppression of thermogenesis, we injected anisomycin and leucocyte pyrogen in a hot environment.

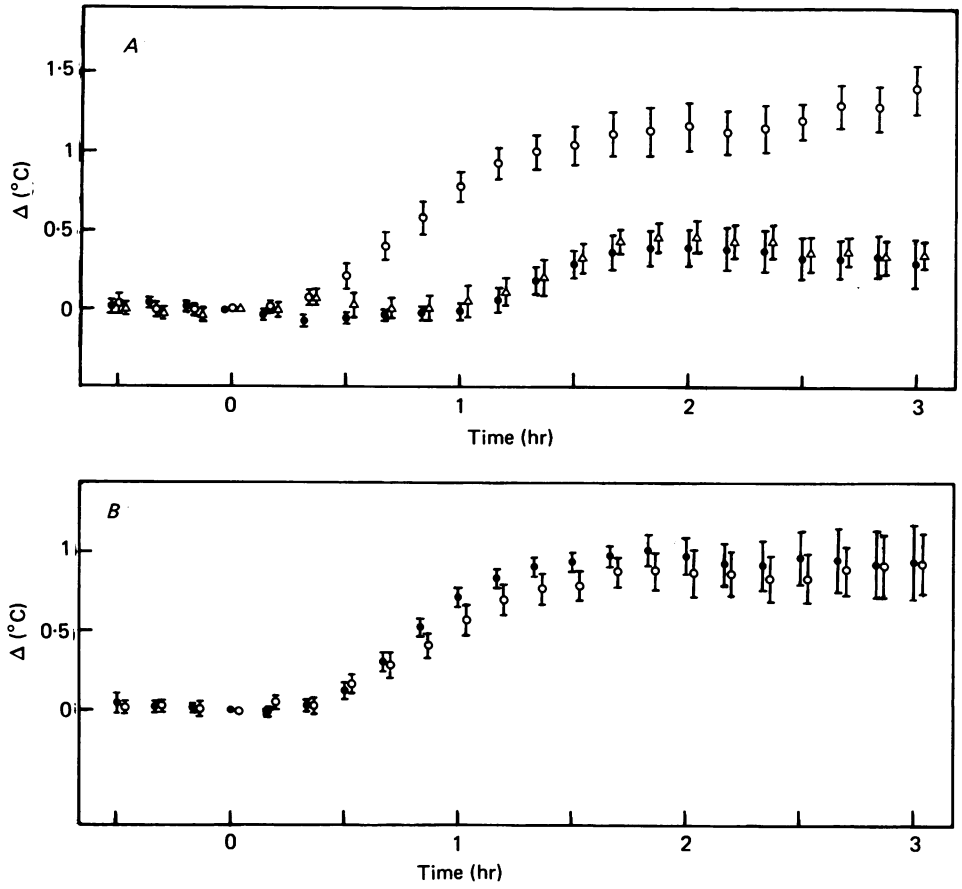

Fig. 4*A*, mean changes in rectal temperature of five rabbits given an I.C.V. injection at time zero of leucocyte pyrogen previously incubated with saline (○), or incubated with anisomycin (●), or both leucocyte pyrogen and anisomycin incubated separately but injected together (△). *B*, mean changes in rectal temperature of six rabbits given an I.C.V. injection at time zero of leucocyte pyrogen incubated with saline and dialysed (○) or leucocyte pyrogen incubated with anisomycin and dialysed (●).

As at normal temperatures, there was a marked inhibition of the fever (See Fig. 3*B*). A comparison of the control fever curves in Fig. 3*A* and 3*B* reveals that the same dose of leucocyte pyrogen produced a smaller fever in the heat. Although we are not able to explain this difference, it is quite clear that anisomycin suppresses fever under hot conditions just as effectively as it does at normal room temperature.

As a further check on the possibility that anisomycin was acting on thermoregula-

tion rather than on pyrogenesis, rabbits were exposed to cold conditions and given I.C.V. anisomycin. There were negligible changes in rectal temperature whether anisomycin or C.S.F. was administered (see Fig. 5).

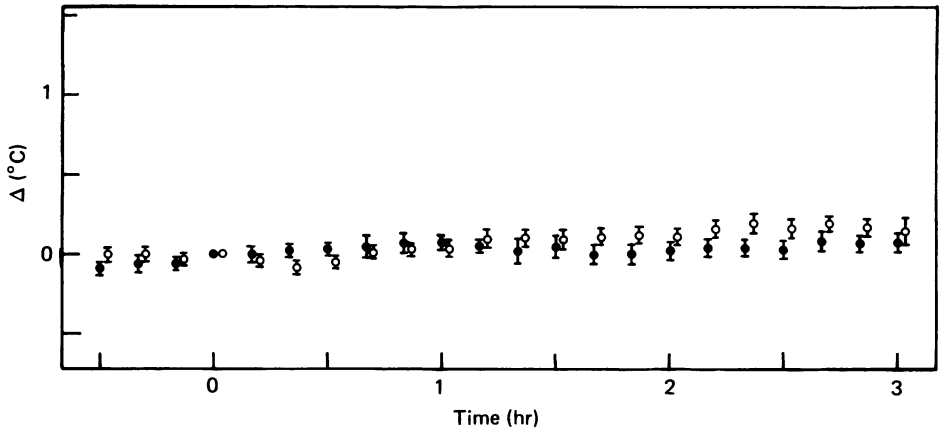

Fig. 5. Mean changes in rectal temperature of five rabbits exposed to room temperature of 9 °C from – 1 hr and given I.C.V. anisomycin (300 μg plus 300 μg/hr) (●) or saline (○) at time zero.

DISCUSSION

The results presented in this paper show that anisomycin given into the cerebral ventricles causes an almost complete inhibition of *de novo* hypothalamic protein synthesis; that the same dose of anisomycin produces nearly total suppression of fever, especially when the pyrogen is also given intraventricularly; that anisomycin does not combine irreversibly with leucocyte pyrogen; that this antipyretic effect is due to a central action of anisomycin; and that a general inhibition of the processes of thermogenesis is unlikely to be the explanation.

In the first experiments in which anisomycin was investigated (Myers, 1980), the pyrogen used was killed *S. typhi*. Although complete suppression of the fever by anisomycin was obtained, the effect could have been due to a failure of the leucocytes to become activated, a process known to involve protein synthesis (Atkins & Bodel, 1971). In the present experiments, this possibility has been avoided by using leucocyte pyrogen.

The same effector mechanisms (viz. thermogenesis and vasoconstriction), are used in fever and in the defence of central temperature against a cold environment. Our evidence shows that only fever was affected by anisomycin, and leads us to infer that the inhibitor must have been acting on some intermediate stage between the initial action of leucocyte pyrogen and the excitation of the thermoregulatory effector neurones.

We have demonstrated clearly that anisomycin has a striking effect on the incorporation of leucine into brain protein. Although there appears to be a close correlation between this effect and the suppression of fever, caution must be exercised in assuming a causal relationship. It remains possible that anisomycin was inhibiting the

febrile response by a central pharmacological action which was distinct from protein synthesis inhibition.

The initial experiments were carried out with a loading dose and continuous infusion of anisomycin, since it was thought that this substance, if acting through inhibition of protein synthesis, might take some time to produce any effect because of the presumed low rates of protein turnover. In the later experiments (Fig. 4*A*, *B*) where for other reasons anisomycin was given simultaneously with leucocyte pyrogen, the suppression of fever was just as effective as in the earlier experiments where anisomycin was given for 2 hr before. If the suppression of fever is really due to interference with protein synthesis, the implication must be that rapid protein turnover can take place in the hypothalamus and that it is important in pyrogenesis.

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