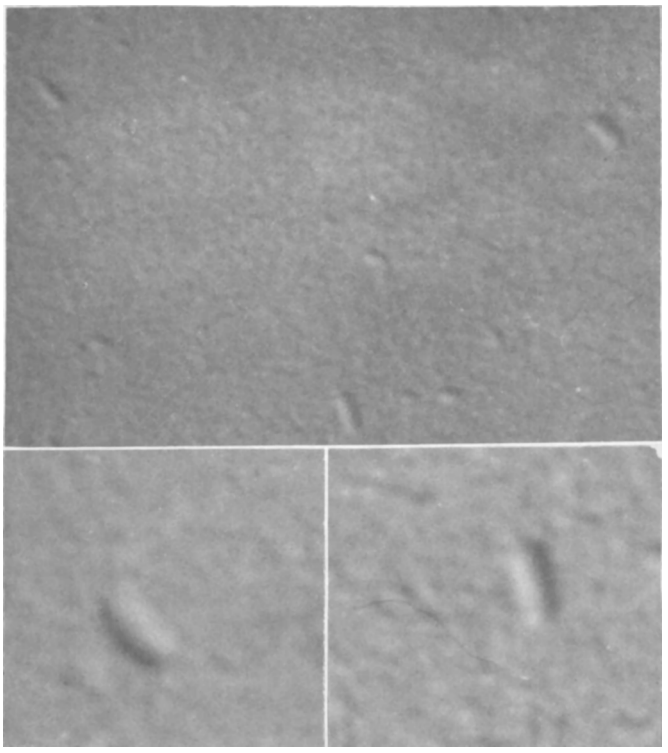

FIG. 1.
Poliomyelitis virus particles (Leon Strain) shadowed with chromium at arc tangent 1/6. Top x 75000; bottom both x 152000.

observation period.

Electron microscope examination of the concentrated virus suspension revealed virus-like particles as shown in Fig. 1. These particles were small and rod-shaped, ranging in width from 12-15 mμ. Upon examination of the concentrated suspension of the normal spinal cord no such virus particles were seen.

Summary. Studies by electron microscopy of the spinal cords of monkeys infected with poliomyelitis virus (Leon strain) showed rod-shaped, virus-like particles ranging in width from 12-15 mμ. These bodies could not be demonstrated in normal monkey cords.

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Comparison of Leukocyte Response to ACTH and Bacterial Pyrogen.* (18672)

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Bacterial polysaccharides, referred to as "pyrogens", induce characteristic alteration of the number of circulating white blood cells.

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Following administration of such material, there occurs marked leukopenia succeeded by marked leukocytosis. These changes have been observed in a variety of species and are sufficiently characteristic of bacterial pyrogens to have prompted the suggestion that they be used as an alternative to the production of fever for the detection of such bacterial products in fluids to be used in human therapy. The alteration of the white blood cell count induced by pyrogen resembles that induced by stress, the administration of ACTH or the administration of adrenal steroids(1-9). In addition, purified pyrogens are reported to elicit tissue changes characteristic of the "alarm reaction"(10). Several observers have interpreted the eosinopenia produced in man by these bacterial pyrogens as evidence of specific pituitary-adrenal activity(11,12,13). However, in the rat, the typical lymphopenia induced by typhoid vaccine is not abolished by removal of the adrenals(14). In view of current interest in agents which are capable of initiating pituitary-adrenal discharge and in specific indicators of such discharge, it was thought worth-while to determine whether the leukocyte response to a purified pyrogen in the dog, which shows eosinopenia in response to ACTH, was or was not due to pituitary-adrenal activity.

This study has revealed that removal of the adrenal substantially reduces the eosinophile response to ACTH, whereas, the eosinophile response to pyrogen is not altered by this procedure.

Methods. Ten normal dogs were anesthetized with pentobarbital sodium. Blood samples were obtained from the femoral vein, a small amount of heparin was added and the total white cells as well as eosinophiles were enumerated in counting chambers. Eosinophiles were prepared for direct count by the eosine-acetone method of Dunger(15). After control counts had been obtained, 5 of these dogs were given 500 μg of either Baxter's P.S. pyromen‡ (prepared from *Pseudomonas aeruginosa*) or P.V. pyromen‡ (prepared from *Proteus vulgaris*)intravenously. The other 5 dogs received 10 mg of ACTH intravenously. The ACTH used was either Armour's 128-105 R§ or 72-50(C).§ The preparation labeled 128-105 R was reported to be 160% of the La-I-A standard. An additional 12 dogs were anesthetized with pentobarbital sodium and bilaterally adrenalectomized through a mid-abdominal incision. These animals were given pyrogen-free saline solution intravenously and 2-3 hours were allowed to elapse prior to collection of blood for control counts. Seven of these dogs were then injected with "pyromen" and 5 with ACTH in doses comparable to the control groups. Following the injection of ACTH or pyromen, blood samples were usually drawn at 5 min., 15 min., 30 min., and at hourly intervals for 6 hours. Total and eosinophile counts were performed in quadruplicate.

Results. Since no gross differences were ob-

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‡ Pyromen supplied through the courtesy of Dr. N. M. Nesset of Baxter Laboratories, Morton Grove, Ill.

§ ACTH preparations supplied through the courtesy of Dr. Irby Bunding of Armour and Co., Chicago.

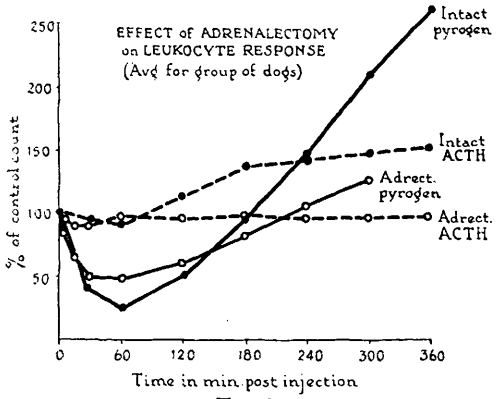

FIG. 1

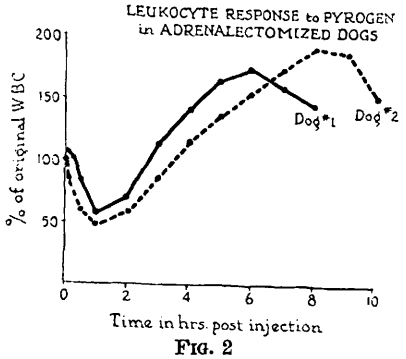

FIG. 2

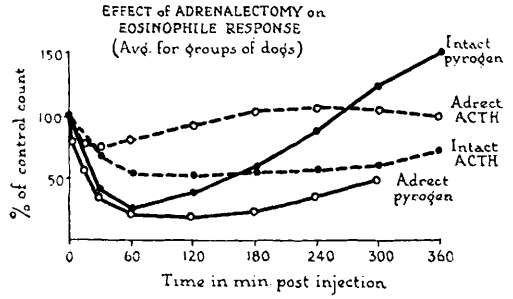

FIG. 3

served in the behavior of the two pyrogen preparations these animals are considered in one category. The same applies to the preparations of ACTH.

The control values for the various groups of animals are as follows: (1) white blood cells per cu mm, intact animals 8240 ± 4367, adrenalectomized animals 9773 ± 3172, eosinophiles per cu mm, intact animals 401 ± 324, adrenalectomized animals 519 ± 588. The response curve for each animal was smoothed by the procedure of running averages and counts were then expressed in terms of the percent of the control values for each animal. Fig. 1 is the average total white blood cell count for each group of animals expressed in terms of per cent of the control counts. It is apparent that, in the doses used, the leukopenia produced by ACTH is only very slight. That produced by pyrogenic material is more marked. Whereas, both pyrogen and ACTH produced some degree of leukocytosis during the period of observation, that produced by pyrogen is more marked.

Adrenalectomy neither abolished nor substantially modified the pyrogen induced leukopenia. ACTH induced leukocytosis was completely abolished by removal of the adrenals. However, the leukocytosis induced by pyrogen, subsequent to adrenalectomy, is somewhat less than that in the control group of animals. It should be indicated that adrenalectomized animals are quite susceptible to the lethal action of pyrogens and all of the present succumbed prior to the sixth hour of observation, at a time when leukocytosis is just beginning to be marked in the normal animal. Fig. 2 is an illustration of the fact that with lower doses of pyrogen (50 μg), marked leukocytosis can be induced in the adrenalectomized dog.

In Fig. 3 it may be seen that adrenalectomy substantially reduces the eosinopenia induced by ACTH. However, eosinopenia induced by pyrogen is, if anything, intensified by adrenalectomy.

The graphs presented are averages of the response of groups of animals. Hence, these composites are somewhat misleading due to the occurrence of maximum effects at varying periods. Thus a table has been prepared in which the magnitude of the maximum response has been reported for each animal without regard to the time of occurrence of this maximum response. From this table it may be observed that the average maximum depression of the W.B.C. count as produced by pyrogen was 76% before and 59% after adrenalectomy. No statistically significant difference exists between these two groups ($p = 0.06$). Correspondingly, the average reduction of circulating eosinophiles was 78% before and 86% after adrenalectomy. These

TABLE I. Extent of Maximum Depression of Circulating W.B.C.

Pyrogen		ACTH	
Adrenalectomized	Intact	Intact	Adrenalectomized
% depression of total W.B.C.			
—45	—83	— 3	— 7
—62	—80	— 1	— 8
—75	—75	—20	—14
—74	—78	— 1	—13
—65	—64	—55	—14
—51			
—38			
Avg —58.6	—76.0	—16.0	—11.2
p 0.06	<0.01	>0.50	
% depression of eosinophiles.			
— 68	—85	—78	—38
— 79	—97	—53	—35
— 82	—83	—83	—15
— 99	—75	—48	—18
—100	—50	—90	—35
— 78			
— 95			
Avg —85.9	—78.0	—70.4	—28.2
p >.40	>.50	<.01	

groups are not significantly different ($p = >0.40$).

It may also be observed that the leukopenia induced by ACTH is significantly less than that produced by pyrogen ($p = <0.01$). However, the eosinopenia induced by ACTH is as great as that induced by pyrogen.

In contrast to the experiments with pyrogen, the maximum eosinopenia induced by ACTH is significantly reduced by adrenalectomy ($p = < 0.01$).

Discussion and conclusions. It is apparent from these studies that certain differences exist between the leukocyte response to ACTH and that to pyrogen. The typical eosinopenia induced by ACTH is mimicked by the injection of bacterial polysaccharides in the normal dog. However, adrenalectomy substantially reduces ACTH induced eosinopenia without modifying that induced by pyrogen. Thus, pyrogens are capable of producing these effects by mechanisms independent of pituitary-adrenal discharge. Such observations cast doubt upon the specificity of eosinopenia *per se* as an indicator of such discharge, and indicate that knowledge of the pyrogenicity of agents purported to induce adrenal discharge is desirable.

The failure of adrenalectomy, in these experiments, to completely abolish the eosinophile response to ACTH in the dog is in contrast to the findings of others(16), and raises the question as to whether the ACTH preparations employed here may not have been contaminated with bacterial polysaccharides. These preparations were tested for their fever producing potentialities in the dog and found to be free of such. However, it should be stated that in this animal moderate leukocyte responses can be obtained with non-pyrogenic doses of bacterial polysaccharides, and further, that in the anesthetized dog, maximum leukocyte responses without fever are the rule.

An additional difference between the leukocyte response to pyrogen and that to ACTH lies in the lack of substantial leukopenia following ACTH in doses sufficient to produce eosinopenia equal to that induced by pyrogen. This supports the contention that pyrogen is capable of its characteristic alteration of the number of circulating white blood cells by mechanisms other than adrenal discharge.

The present evidence suggests that the extent of the leukocytosis produced by pyrogen is somewhat less in the adrenalectomized than in the intact dog (Fig. 1). A similar observation has been made on the rat. The leukocytosis following typhoid vaccine appears to be less in this species following adrenalectomy(14). From this it might be inferred that leukocytosis following pyrogen is partially dependent upon adrenal stimulation. However, in our experience, as well as that of others(17), the adrenalectomized animal has a tremendously increased susceptibility to the lethal action of these bacterial products and these moribund preparations may be incapable, for non-specific reasons, of a sustained increase in leukocyte production. In partial support of this notion is the observation that smaller, and more readily tolerated, doses of pyrogen do give rise to sustained leukocytosis (Fig. 2).

Summary. The leukocyte response to bacterial pyrogen is superficially similar to that produced by ACTH. However, removal of

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the adrenals substantially modifies the response to ACTH in the dog, but is without effect on pyrogen induced leukocyte responses. It is concluded that pyrogen produces its characteristic changes in the W.B.C. count by mechanisms other than pituitary-adrenal discharge.

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Anti-Tubercular Activity *in vivo* of Nicotinaldehyde Thiosemicarbazone and Its Isomers. (18673)

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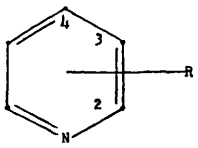
The anti-tubercular activity of 4-acetylaminobenzaldehyde thiosemicarbazone (Tibione) has been well established in experimental infections(1-5). The extensive chemical variations in the series of thiosemicarbazones has not yet resulted in chemically related compounds of appreciably higher activity(6-8), although the ethyl sulfonate of Tibione appeared somewhat superior to the parent substance in the studies by Hoggarth and Martin (4,8). These authors(4) also mention one heterocyclic compound of marked activity: quinoline-4-aldehyde thiosemicarbazone. The recent observations of Levaditi and his coworkers(9) according to which the thiosemicarbazone of nicotinaldehyde exerted a marked effect in experimental tuberculosis of mice seemed to offer a new approach to chemotherapeutic agents with anti-tubercular activity. Levaditi's findings are in agreement with the results of studies with compounds of this type carried out in our laboratories during the last year. This work was undertaken in connection with the studies of Chorine(10) and of McKenzie *et al.*(11) on the effect of niacinamide on experimental tuberculosis. While we were able to confirm the activity of this substance, we failed, as had McKenzie and his group(12) to find derivatives of niacinamide which were superior to the parent substance, until the thiosemicarbazone derivatives were synthesized in the Roche Chemical Laboratories. Of the numerous compounds synthesized by Drs. H. H. Fox, T. S. Gardner, F. A. Smith and E. Wenis and investigated during recent months, the biological properties of the following three substances will be described: 1. the thiosemicarbazone of picolinaldehyde (I); 2. the thiosemicarbazone of nicotinaldehyde (II), and 3. the thiosemicarbazone of isonicotinaldehyde (III). Tibione was used as the reference substance.



$R = CH{=}N{-}NH{-}\overset{\overset{S}{\|}}{C}{-}NH_2$

I in 2—position
II in 3— ''
III in 4— ''

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