of the standard Chambers' micromanipulator at the junction of the pin hinge. The original coarse vertical adjusting mechanism was also removed. The upright piece (F) of the horizontal movements was attached to the free edge of the Dural block (B) with 2 machine screws.

The horizontal movements (E) mounted on the vertical control mechanisms (A) are supported by right-angle, reinforced brackets (C), 2.2 cm wide and 11.0 cm high, made from brass 0.6 cm thick. A bracket 11.0 cm high, with the vertical control mechanism (A) attached as shown in Fig. 2, is ideal for microscope stages approximately 12.5 cm high. For microscopes with lower or higher stages, the height of the supporting bracket should equal the height of the stage minus 1.5 cm. The bases (D) of the supporting brackets, measuring 4.0×4.5 cm, were attached with machine screws to the micromanipulator base plate (G) in front of the microscope.

The microneedle holder clamps (H) are securely held by the micromanipulator with a single set screw (not shown in the figures).

The finished instrument is neat, stable, and correctly adapted to the microscope. There are several advantages provided by this new instrument, which has now been in service more than 2 years. *First,* the movements of the microtips in the vertical axis are rectilinear, and thus the crossline reference technique may be properly used. *Second,* the rack-and-pinion coarse movement is convenient for adjusting the preliminary height of the microneedle or micropipette over a range of several centimeters. It is essential that micromanipulators be equipped with mechanically controlled coarse vertical adjustments so that the microneedles or micropipettes may be quickly moved up or down (*6*). Mechanically controlled, coarse adjustments for the horizontal controls are unnecessary, since the preliminary settings of the microinstruments may be accomplished adequately by freehand under low magnification. *Third,* the fine adjustment of the vertical controls permits the movement of microinstruments into or out of operating position with considerable precision. The range of this movement, sufficient for any occasion, is about 0.3 cm. *Fourth,* micromanipulators equipped with this type of vertical control may be adapted equally well either to conventional microscopes or to the inverted microscope (*6*).

The instrument described in this article was made by the Gamma Scientific Company, from whom the standard Chambers' micromanipulator may also be obtained.

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Constitutional Factors in Resistance to Infection: The Effect of Cortisone on the Pathogenesis of Tuberculosis[1]

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In an endeavor to identify the constitutional factors responsible for the genetic resistance and susceptibility to tuberculosis of highly inbred rabbit races, it was found that the administration of estrogen to susceptible rabbits materially increases their resistance, whereas the periodic exposure of resistant rabbits to chorionic gonadotropin enhances their susceptibility to the disease (*1, 2*). It was noted in this report that tuberculosis in rabbits is accompanied by a marked hypertrophy of the adrenal cortex. In later studies it was observed that the degree of hypertrophy of the adrenal cortex of natively resistant rabbits affected by tuberculosis is much greater than that of susceptible rabbits similarly infected; therefore, investigations on the role of the adrenal cortex in resistance to the disease were begun. The general plan of the undertaking is to determine whether by increasing the adrenal function resistance can be increased and, conversely, whether by lowering this function the native resistance can be diminished.

Using cortisone, which is one of the important hormones of the adrenal cortex in synthetic form, the following experiment was performed. Twenty littermates of the genetically uniform and highly susceptible strain, FC, were divided into two groups of 10 each. They were placed in a room at a constant temperature of $21 \pm 2°$ C. Their urine was collected for analysis of the contained steroids. Total and differential counts of blood cells and fasting blood sugar were determined. At the same time the spread of India ink and of rabbit hemoglobin in the skin was measured 4 hr after injection. The inflammation at the site of injection of these substances in the skin was ascertained on the next day. After these base lines were established, 10 of the rabbits received 2 mg cortisone acetate per kg, intramuscularly, on alternate days. The 10 control littermates received the same volumes of the suspending medium without the cortisone by the same route, at the same intervals. Three days after the beginning of cortisone treatment, when the absolute number of circulating lymphocytes in the blood of the experimental animals had been markedly depressed, and when the fasting blood sugar of the same animals had increased by comparison with the essentially unchanged levels of these items in the control animals, both groups were simultaneously exposed to the quantitative inhalation of known numbers of viable, viru-

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lent, human-type tubercle bacilli, H 37R v, in the apparatus for experimental airborne infection, which has been carefully evaluated as previously described (*3*, *4*).

After the control and experimental animals were infected, the cortisone and the suspending medium, respectively, were administered to each group at the same intervals and in the same amounts, as stated above, throughout the course of the experiment. During this time the absolute number of circulating lymphocytes of the blood, the fasting blood sugar, the blood ascorbic acid, the development of tuberculin sensitivity and antibodies against the tubercle bacillus, the spread of India ink and rabbit hemoglobin in the skin, and the inflammation induced by these agents in this tissue were measured. At this time the excretion of steroids in the urine was also determined.

It was found that the absolute number of circulating lymphocytes of the cortisone-treated animals was continuously diminishing, in statistically significant amounts, with continuation of treatment, whereas that of the controls actually increased as compared with their respective base lines. Likewise, the fasting blood sugar of the experimental animals increased with the duration of treatment, whereas in the controls this increment was much less. Definite amounts of ascorbic acid were found in the blood of the controls, whereas in the experimental animals this substance had either completely disappeared or remained only in traces. The spread of India ink or hemoglobin in the skin was not definitely affected in either the control or experimental animals. However, the inflammation induced by these agents in the skin was noticeably reduced in the experimental as compared with control animals, and in statistically significant amounts. This is clearly due to the reduction in the permeability of the vessels induced by cortisone, as demonstrated by Menkin (*5*). Similarly, the tuberculin reaction in the skin was markedly suppressed in the cortisone as compared with the control rabbits. The effect on antibody production was not conspicuous, although the titer in the experimental was slightly though uniformly lower than in the controls.

In view of the demonstration by Gordon and Katsh (*6*) that starvation, by stimulating the adrenal function, increases the phagocytic activity of the macrophages, half the control and experimental rabbits were given 6 ml of a 1:3 dilution of India ink in saline per kg intravenously, 3 hr before they were killed 34 or 36 days after infection. Their livers and spleens were digested with concentrated KOH, and the weight of carbon present in the spleens and livers was determined. It was found that the phagocytosis of carbon particles by these organs was markedly increased in

TABLE 1

EFFECT OF CORTISONE ON RATIO BETWEEN NUMBER OF HUMAN-TYPE TUBERCLE BACILLI INHALED AND NUMBER OF PULMONARY TUBERCLES GENERATED IN FC RABBITS, 34–38 DAYS AFTER INFECTION

Controls					Cortisone-treated				
Rabbit No.	No. TB estimated as inhaled	No. tubercles in both lungs and their size		No. viable bacilli yielding 1 tubercle	Rabbit No.	No. TB estimated as inhaled	No. tubercles in both lungs and their size		No. viable bacilli yielding 1 tubercle
		No.	Diam (mm)				No.	Diam (mm)	
FC2 = 46 (C 11)	4,470	51	6	88	FC2 = 1 (E 1)	6,258	165	2	38
FC2 = 47 (C 12)	5,960	63	5	95	FC2 = 49 (E 2)	5,662	142	2	40
FC3 = 18 (C 13)	5,960	*	5		FC3 = 24 (E 3)	5,066	*	3	
FC2 – 50 (C 14)	6,647	72	5	92	FC2 – 51 (E 4)	6,647	275	3	24
FC3 = 29 (C 15)	7,233	32	5	226	FC3 = 30 (E 5)	7,038	131	4	54
FC3 = 31 (C 16)	1,796	17	6	105	FC3 = 32 (E 6)	1,802	40	3	45
FC4 = 3 (C 17)	2,120	8	Large	265	FC4 = 2 (E 7)	1,802	71	Small	38
FC3 = 37 (C 18)	1,590	36†	5	44	FC3 = 35 (E 8)	1,378	36	"	38
FC3 = 36 (C 19)	1,440	9	6	160	FC3 = 39 (E 9)	1,440	42	2	34
FC2 – 56 (C 20)	1,080	11	7	98	FC2 – 55 (E 10)	1,440	40	1	36
			Av	130 ± 68				Av	37 ± 9

* These lungs are to be photographed; the number of tubercles they contain will be determined later.
† Eighteen of these tubercles were barely visible.

the cortisone-treated animals. The remaining rabbits were killed 35 or 38 days after inhalation. In all cases, each cortisone-treated rabbit was killed on the same day following infection as its control littermate that had inhaled the same infected air, for the same time, at the same sitting. All animals were starved for 17 hr before they were killed by air embolism. The glycogen present in the livers of all control and experimental animals was determined and the weights of the liver, spleen, adrenals, gonads, and pituitary were ascertained.

It was found that the livers of the cortisone-treated rabbits were twice as heavy as those of the controls. This was due to large deposits of glycogen and fat in the liver cells of the experimental animals from the physiological effects of the cortisone. The spleens of the cortisone-treated rabbits were markedly reduced in weight, obviously as a result of the lympholytic effect of cortisone. The adrenals were markedly atrophied in the experimentals, clearly due to the suppression of the secretion of ACTH by the pituitary, produced by the high level of cortisone in the blood. There was also marked atrophy of the male gonads, though the ovaries were not affected. The thyroids of the experimental rabbits were uniformly though only moderately reduced in weight.

It is clear from these results that the experimental animals were under the intense physiological effects of cortisone, though this cortisone had also apparently reduced the remaining functions of the adrenals and other glands of internal secretion.

The number and size of the tubercles developed in the lungs of the control and experimental rabbits were accurately determined and are listed in Table 1. It will be noted that the number of tubercles generated in the cortisone rabbits was uniformly greater than in the controls. On the average, four times as many tubercles resulted from the inhalation of human tubercle bacilli in cortisone-treated rabbits as from the inhalation of the same numbers of bacilli by untreated littermates, of the same inbred strain and the same genetic resistance to the infection. This difference is statistically significant.

However, the size of the tubercles in the lung, and their spread, underwent uniform and striking reduction in the cortisone-treated animals. Furthermore, the spread of the disease to the draining tracheobronchial lymph nodes and apparently, also, to the internal organs was markedly reduced in the experimental rabbits. The caseation in the tubercles of the cortisone-treated rabbits was greatly increased and compact as compared with that of the control animals. Further analysis of these differences is now in progress and will be reported in a subsequent publication, together with a detailed exposition of the data summarized in this paper.

It is clear from these results that cortisone has markedly and fundamentally affected the essential mechanism of the pathogenesis of tuberculosis. We have previously reported (*7*) that the highly genetically resistant rabbit family A, when exposed simultaneously to tuberculous roommates with the members of the highly susceptible F family, acquires tuberculosis, on the average, 3 months after the beginning of the exposure. The F rabbits, on the other hand, acquire the disease in 6 months. Whereas, however, the A rabbits die from a chronic, localized, ulcerative pulmonary phthisis 8 months after the onset of the disease, the F rabbits die from a rapidly spreading, generalized tuberculosis 4 months after the inception of the tuberculosis. Resistance to infection is, therefore, two-phased. Resistance to attack by natural airborne infection is distinct from resistance to the progress of an already engrafted disease. It is clear from the above that cortisone has increased the engrafting of the tubercle bacilli in the lungs of these susceptible animals, just as the natural infection behaves in resistant rabbits. Likewise, cortisone has retarded the dissemination of the disease in susceptible rabbits, as occurs in resistant rabbits. The simplest explanation of these observations is the fact that cortisone increased the phagocytic activity of the reticuloendothelial cells and hence, also, of the alveolar macrophages. More of the inhaled bacilli will, therefore, be retained in the alveolar phagocytes of the cortisone-treated rabbits than in those of the controls, and consequently more tubercles will be generated in the lungs of the former.

Whether the greater extent of the caseation observed in the tubercles of the cortisone-treated rabbits is due to a greater destructive capacity for tubercle bacilli afforded the macrophages by the hormone, or to the reduced permeability of these cells and, therefore, to a greater concentration of the allergically toxic derivatives of the tubercle bacillus in the experimental animals, remains to be determined. In any event, it has been demonstrated that cortisone can transform a rabbit of high genetic susceptibility to the disease into one that shares some of the essential pathological responses characteristic of the natively resistant rabbit.

Addendum. Since the above was submitted for publication, microscopic study has revealed that in the cortisone rabbits the tubercles were sharply delimited, caseous pneumonic foci with bacilli swarming in the necrotic alveolar plugs. There was little interstitial inflammation about them. In the controls the tubercles were essentially widely spreading, interstitial tuberculous granulomas in which the bacilli were much less numerous. The caseation in these was often discrete, less extensive, but further advanced than in the cortisone group. The hormone, instead of increasing the destruction of the bacilli, as was inferred above, actually greatly increased their numbers in the lesions. Cultural studies of the lungs and lymph nodes of the cortisone-treated and control rabbits were done immediately after, and 2 weeks after inhalation. It was found that, notwithstanding the greater phagocytic activity of the reticuloendothelial cells of the treated animals, no greater deposition of tubercle bacilli occurred in the lungs of the experimental animals. However, 2 weeks after infection, despite the far greater number

of living tubercle bacilli in the lungs of the cortisone rabbits, their dissemination to the draining tracheobronchial lymph nodes was very much less than in the control animals.

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The Adaptive Increase of the Tryptophan Peroxidase-Oxidase System of Liver

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The activity of the recently discovered system in liver converting tryptophan to kynurenine (*1*) increases following the administration of tryptophan and certain other substances to an animal. This increase is referable to the tryptophan peroxidase-oxidase system, which is an example of a physiological "coupled oxidation" (*2*): a peroxidase reaction specific for L-tryptophan followed by a second oxidation to formyl-kynurenine, using oxygen and producing hydrogen peroxide for the first reaction. The change of this system in response to treatment of the animal suggests that one of the mechanisms in animals for control of metabolism by alteration of enzyme activities may be analogous to the enzyme adaptation of microorganisms.

This tryptophan oxidizing system could not be found in normal animals until a sensitive assay was available, and was originally demonstrated in the livers of rabbits given tryptophan for the purpose of isolating kynurenine. A low activity can be demonstrated in normal rabbits, but rabbits given 4 g of L-tryptophan the previous day are frequently found to have activities up to ten times those of normal animals. The magnitude and reproducibility of this adaptive increase is shown in Tables 1 and 2. The activity of the system is determined by the formation of kynurenine from L-tryptophan in the supernatant of a fresh liver homogenate provided with adequate amounts of enzyme-generated hydrogen peroxide (*1*). The formyl-kynurenine produced by the coupled oxidation is hydrolyzed to kynurenine by the enzyme formylase. Formylase is present in liver preparations in a 600-fold excess over the coupled oxidation reaction, so that increased kynurenine formation by the system must be due to increased activity of the limiting oxidizing steps. The formylase, measured by an independent method depending upon the hydrolysis of formyl-anthranilic acid (*3*), does not change significantly in preparations showing over tenfold increase in the oxidizing activity (Table 1).

TABLE 1

Treatment	No. animals	μM kynurenine/g Liver/hr		Formylase
		Average ± P.E.	Range	Average ± P.E.
Rabbits				
Normal	9	1.4 ± 0.3	0.90 – 2.10	84 ± 22
5–12 hr after 10 mM DL-tryptophan	10	11.7 ± 1.3	9.60 – 15.30	104 ± 20
15–20 hr after 10 mM DL-tryptophan	8	2.7 ± 0.6	1.80 – 4.20	—
Rats				
Normal	22	1.2 ± 0.2	0.81 – 1.50	—
4–10 hr after 2–4 mM L-glutamic acid, NH_4Cl or DL-alanine	10	1.5 ± 0.3	0.64 – 2.22	—
4–10 hr after 1–2 mM DL-tryptophan	7	8.0 ± 1.8	5.65 – 12.30	—
5–8 hr after 1–2 mM L-tyrosine or L-phenylalanine	4	4.4 ± 0.5	3.84 – 4.56	—
4–8 hr after 2 mM L-histidine	7	9.3 ± 1.4	5.29 – 11.80	—

TABLE 2

Hr after 2 mM L-histidine	No. rats	μM kynurenine/g liver/hr	
2	2	2.3	2.5
4	2	8.4	9.0
5.2	1	11.8	
7.8	2	10.4	11.3
12.5	2	1.9	2.5

Several alternative explanations for this change in the tryptophan oxidizing activity upon tryptophan administration have been tested. The livers of treated and untreated animals are similar in weight and water content. The increased activity after treatment may be demonstrated in liver slices as well as in extracts. The kynurenine formed by the enzyme blanks (without tryptophan) is negligible, even after tryptophan administration to the animal. The enzymes extracted from both types of animals are qualitatively the same. In neither is there evidence of a dissociable co-factor for the system. The activity of a combination of normal plus adapted enzymes is a simple addition of their activities separately. Only liver, and not other tissues, contains the system. The known animal peroxidases from milk and from white blood cells do not augment the reaction of the liver peroxidase system. These observations rule out, as explanations of the increase in activity, various possibilities such as preservation or increased extractability of the enzyme, accumulation of an intermediate, provision of a dissociable co-

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