found in the adrenal medulla of the rat and in the hormone-treated group these medullary cells were likewise characterized by unusually coarse lipid granules. This change was not observed in animals treated with steroids devoid of testoid properties. It will be recalled that folliculoids cause hypertrophy of the cortical cells accompanied by an actual loss of lipid granules while corticoids induce a generalized atrophy of the cells in all 3 layers of the cortex; thus the response of the adrenal to treatment with testoids appears to be rather specific.

The influence of the adrenal cortex on potassium metabolism is well known and in connection with the adrenal changes described above it is of interest to note that Butler *et al.*[8] observed a pronounced fall in the serum potassium of patients treated with methyl-testosterone. We therefore determined the serum potassium concentration (method of Kramer and Tisdall[9] modified by Jacobs and Hoffman[10]) of our rats on several occasions during the course of the experiment. However, in this respect there was no statistically significant difference between the two groups.

It is also of interest to note that in spite of this long continued treatment with high doses of methyl-testosterone, these animals showed no other manifest signs of damage except for a slight inhibition of somatic growth, their average final body weight being 196 g while that of the controls was 236 g.

Summary. Experiments on male albino rats indicate that chronic treatment with large doses of methyl-testosterone elicit changes in the adrenal cortex which are characterized by an involution of the glomerulosa, marked thickening of the connective tissue and deposition of coarse fat granules in the cells of the fasciculata and reticularis. In many cases these fat granules fuse into a single voluminous lipid globule which distends the cytoplasm and pushes the nucleus to one side. Consequently the epithelial cells of the cortex assume the appearance of ordinary fat cells. Although in the rat testoid compounds do not cause a specific involution of the reticularis as they do in the mouse, the above mentioned changes are considered to be a typical response of the cortical cells to overdosage with testoid substances.

Contrary to previous observations in man the serum potassium showed no significant change following treatment with methyl-testosterone in the rat.

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8 Butler, A. M., Talbot, N. B., and MacLachlan, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 378.

9 Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **46**, 339.

10 Jacobs, H. R. D., and Hoffman, W. S., *J. Biol. Chem.*, 1931, **93**, 685.

14339 P

Use of Bacterial Asphyxia for Homologous Antigen Production in Experimental Immunization Against Human-Type Tuberculosis.*

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In tuberculosis no wholly satisfactory immunity of man and domestic animals has been obtained. This may be due in part to the unsatisfactory constitution of the vaccines employed. It is therefore restated that human, bovine, and avian type bacilli may be killed

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without the use of coagulating heat or of chemicals, by incubation at 38-40°C in a partial vacuum, saturated with water vapor but deprived of oxygen.[1] In this condition the bacilli are evidently forced to metabolize in the absence of a new supply of one of their most essential constituent elements. Perhaps they are "sabotaged" by their own internal enzymes but oxidation of the protoplasmic substance is inhibited by the absence of molecular oxygen. The death of these bacilli, from the sequelæ of oxygen deprivation in a moist warm state, I have called, for lack of a better term, asphyxia. Various investigators have communicated a scattering of published or unpublished observations suggesting that many, if not all, disease parasites might be killed in a somewhat similar fashion. Some adaptations of the technic to deprive the anaerobes of combined oxygen, to fit the time and temperature of incubation to the delicacy of particular parasites, and to remove exotoxins after killing, appear indicated for other organisms. Yet in general the application of parasitic asphyxia to the needs of immunology appears to be a wide and virtually untried field.

Marked protection against avian tuberculosis of the rabbit, by a vaccine killed in an essentially similar fashion (incubation of glass-sealed cultures), has previously been reported.[1] However, it could not be assumed from these observations on the avian bacillus that the human-type organism would also autoasphyxiate itself without irreparable mischief to its specific immunizing power. Also it seemed desirable to speed up the killing for these tough vegetative parasites by immediate elimination of oxygen and medium from the glass-sealed tubes.

In the present experiments, therefore, pyrex glass tubes each containing 0.5 cc boiled tap water† and the growth removed from 10 one-month-old glycerine-agar cultures of human tubercle bacilli (strain H63) were prepared. The tubes were evacuated to a reading of 2 cm pressure, degassed, washed out repeatedly with hydrogen, evacuated, and glass-sealed at about 2 mm residual pressure of water vapor. A few drops of liquid water remained in each tube. The contents were then sterilized by incubation for one month at 40°C. Death of the bacilli was confirmed by attempted culture on Petragnani medium, and by attempted infection of 16 guinea pigs and 8 hamsters.

For immunization, the rabbit was selected as an experimental animal. Large intravenous doses (0.1-0.5 mg) of virulent human strains, in rather coarse suspensions, produce in this animal a largely self-limited disease characterized by extensive morbidity but low mortality.

Litters of 4-6 rabbits each, free from coccidiosis and snuffles, were selected. Each litter was derived from animals inbred, brother and sister, for 3-4 generations. Sixty-six rabbits in all, in 2 series, were employed. One-half of each family was reserved for controls.

The other half of each family was immunized by 6 weekly subcutaneous injections, partly in each quadrant, of approximately 13 mg each of the asphyxiated vaccine. A portion of each thick, milky suspension of the bacilli was tested at the same time for any residual virulence by inoculation in the guinea pigs and hamsters. Four months after immunization all living rabbits were intravenously inoculated with virulent bacilli. Three doses ranging from 0.1-0.5 mg were employed in different groups.

Two control animals and no immunes died prior to test injection with living bacilli, apparently from heat prostration. The immunes, although young 4-lb animals at the start, were but little retarded in weight gain as compared with the controls by the heavy doses (totaling 78 mg) of vaccine they received. After test injection of living bacilli, 4 controls and 3 immunes died at 3-6 months; these deaths could not be definitely ascribed to tuberculosis alone; they were due in part to heat prostration. At 8-10 months the remaining animals were killed in pairs. One whole family of 6 animals inoculated with the smallest dose showed at this time no gross lesions in either controls or immunes. All other controls, except 2, showed gross lesions, often extensive, of the lungs, kidneys, oviducts,

1 Potter, T. S., *J. Infect. Dis.*, 1942, **71**, 220, 232; *Fed. Proc.* (Imm.), 1943, **2**, 101.

† Phosphate buffer solution may prove a distinct improvement.

TABLE I.

	Controls	Immunized
No. of rabbits at start	33	33
Died before test infection	2	0
Died (in heat wave)	4 (3 showed lesions)	3 (1 showed lesions)
Resistant family showing no lesions	3	3
Others showing no lesions at 8-10 months	2	24
Total showing lesions at 8-10 months	22	3

testes, mammary glands, bones, or subcutaneous lymph nodes. Microscopic sections of these lesions showed frequently fuchsinophile detritus with only a few intact bacilli. The immunes, except 3, totally escaped such lesions; when present lesions were few, and were small ones of the kidneys or subcutaneous lymph nodes.

Comment. The best experimental results on immunization against tuberculosis have been obtained previously by use of living tubercle bacilli heterologous in type, such as BCG,[2] and with heat-killed bacilli, homologous in type.[3] In sharp contrast to the results obtained in immunization with whole bacilli, living or dead, I have found to date no record of successful results with chemically fractionated bacilli. Satisfactory comparison between the living and non-living vaccines is difficult because the living persist longer and doubtless multiply for a time thus producing more numerous and persistent foci of stimulation than so far obtained with killed organisms. For purposes of comparative study the largest well tolerated doses rather than equal doses might well be employed. The killed and non-multiplying vaccines present the advantages of using the very type of parasite one wishes to immunize against, and of superior control of the final quantity, distribution. and persistence of the antigen.

The results here submitted and those previously presented confirm the conclusion of Opie and Freund[3] that a considerable protection of the rabbit is possible by use of completely killed tubercle bacilli as a vaccine. Protection of the guinea pig‡ has also been observed with asphyxiated bacilli but less complete than that obtained simultaneously with equal doses of BCG, and less than that of the rabbit. As pointed out by Opie and Freund the rabbit diseases resemble human tuberculosis in virulence far more than the highly fatal guinea pig infections. (*Cf.* high incidence of tuberculin reaction in people offering no clinical symptoms, and the low death rate even in sanatoria). Avian swine tuberculosis is also a disease of moderate virulence.

Due to limitations in animal space no conclusive comparative work between heat-killed and asphyxiated bacilli has been completed to date. The doses of asphyxiated vaccine used in rabbits were approximately 30 times those of heat-killed vaccine employed by Opie and Freund and 300 times those of BCG clinically advisable. The human test strain of bacilli employed by me was less virulent than the Ravenel bovine strain employed by Opie and Freund, but the test doses were approximately 10,000 times as great and the protection obtained apparently more complete.

Summary and Conclusion. Following the immunization of 33 rabbits with tubercle bacilli killed by asphyxia, 29 of the animals showed (mostly at 8-10 months) no tuberculous lesions. Among 31 normal siblings of the above 25 exhibited gross tuberculous lesions. Thus parasitic asphyxia is a flexible and virtually untried principle for use in immunology. It appears promising for study in human-type as well as in avian-type tuberculosis.

[2] Townsend, J. G., Aronson, J. D., Saylor, R., and Parr, I., *Am. Rev. Tub.*, 1942, **45**, 41.

[3] Opie, E., and Freund, J., *J. Exp. Med.*, 1937, **66**, 761.

‡ Low toxicity of the whole asphyxiated bacilli for guinea pigs was demonstrated.