on constant, weighed diets, and careful metabolic technique was followed throughout. Studies were made of glucose tolerance, urinary excretion of sodium, potassium, chloride, phosphorus, nitrogen and 17-ketosteroids. With the possible exceptions of a slight increase in potassium excretions in two subjects, a transient rise in 17-ketosteroid excretions in one subject and moderate decrease in tolerance for glucose in two, the findings pointing towards changes in the adrenal cortex were negative. Since all these subjects developed an impairment of peripheral vision at altitude, there would seem to be no relationship suggested between the alteration in peripheral vision and any of the variables studied by Bryan and Ricketts.

Certain lines of evidence point to the cortex of the frontal lobes of the brain as the probable region involved in the alteration of peripheral vision described here. A similar impairment has been found to result from removal of one or both prefrontal lobes in neurosurgical patients studied in this laboratory, while the effect has not been found following unilateral removal of any other lobe of the brain.[5] The effect has also been found here to occur on a transitory basis following partial destruction of both frontal lobes in man by the operation known as lobotomy. In this connection it is of interest that Kennard[6] and others have found a temporary alteration of peripheral vision to be produced by localized lesions in the frontal lobe of the Macaque monkey.

In the present investigation, 65 per cent. of the subjects exposed to chronic intermittent anoxia of altitude pressures as low as 10,000 feet above sea level developed a marked impairment of peripheral vision. It seems clear that former Service regulations which specified the use of oxygen above this altitude did not provide an adequate margin of safety.

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INHIBITION OF GROWTH OF MYCOBACTERIUM TUBERCULOSIS BY A MOLD PRODUCT[1]

A NUMBER of reports have been made on the effect of various antibiotic agents derived from molds and higher bacteria upon the growth of *M. tuberculosis in vitro* and in some instances on the inhibition of experimental animal tuberculosis.[2,3,4,5,6,7] The present investigation is concerned with the *in vitro* inhibition of the tubercle bacillus by another product obtained from an as yet incompletely identified mold, one of a group of Aspergillaceae under study at the present time. The mold was grown on a modified Czapek-Dox medium for 14 days, the medium filtered clear of mold and debris, acidified to pH 1–2 and extracted with ether. The active material was separated from the ether by 0.5 N NaOH. Concentration was accomplished by repeated ether-alkali extractions using decreasing quantities. The final alkali extract, representing a concentration of the production medium of from 50 : 1 to 300 : 1, was neutralized with 4 N HCl. A highly colored product was obtained. This was found to be soluble in water or alkali and insoluble in acid. The acid precipitate was readily soluble in ether, chloroform or ethyl acetate.

The rapidly growing non-pathogenic strain of *M. tuberculosis* var. *hominis* (American Type Culture Collection No. 607) was used to test the antibiotic activity of the preparation. A standard inoculum, when floated on the surface of Long's or Dorset's synthetic liquid medium and incubated at 37.5° C, shows rapid growth so that within 5 days a dense, crinkled pellicle covers the surface of the medium and extends up the sides of the test-tube for a height of about 0.5 cm. A series of test-tubes, 1 inch in diameter containing 5 ml of liquid medium, to which varying concentrations of extract were added, was inoculated with a standard fragment of pellicle obtained from a freshly growing culture. The tubes were incubated at 37.5° C for from 5 to 14 days and the variation in growth recorded daily. It was found that the inoculum failed to show any evidence of proliferation when the concentration ranged from 0.03 to 0.09 ml of extract per ml of liquid medium, depending upon the potency of the extract. Partial inhibition of growth, which was characterized by varying degrees of proliferation of the inoculum, was obtained in a range of 0.01 to 0.04 ml extract per ml liquid medium. Culture of the partially or completely inhibited inoculum which was still floating at the end of the test period, however, showed active growth when transplanted on solid media. In another series, inocula were submerged in liquid medium to which a quantity of extract had been added that was known to be sufficient to produce complete inhibition of growth of a floating inoculum. Controls consisted of submerged inocula in liquid medium to which no

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extract had been added. After incubation for 5 days at 37.5° C, the organisms were centrifuged, washed with saline and transplanted to a solid medium. Whereas the controls grew out rapidly the organisms exposed to the extract failed to show any growth.

Thirty series of experiments were performed, using the material concentrated from as many separate harvests of mold substrate, representing a total of about 500 liters of original media.

The extract showed little deterioration when incubated for 2 weeks at 37.5° C or when autoclaved at 15 pounds pressure for 15 minutes. A slight lowering of antibiotic activity was noted when incubated with normal human serum or Sorensen phosphate buffer, pH 7.2 to 7.6. No effect on the extract was noted when treated with a Paulitsch borate buffer, pH 7.2 to 7.6.

Penicillin, which has already been shown to have no effect on the tubercle bacillus,[8] was tested against the strain used in our experiments. A concentration of 1,500 units per cc of Dorset's liquid medium failed to inhibit the growth of the standard inoculum used in these studies.

A strain of the human tubercle bacillus, isolated from the sputum of a patient with advanced pulmonary tuberculosis and shown to be highly pathogenic for guinea pigs, was inhibited *in vitro* by the mold extract in preliminary tests. Further experiments now in progress include isolation and purification of the active principle and the determination of the growth conditions necessary for optimum yield of the mold product.

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SULFONAMIDES AND EGG-SHELL FORMATION IN THE DOMESTIC FOWL

THE action of sulfa drugs on egg-shell formation in birds has been reported in papers which appeared in the last two years. Hinshaw and McNeil[1] first noted the laying of soft-shelled eggs by turkeys and White Leghorn hens following feeding of sulfanilamide. Scott *et al.*[2] published a more comprehensive study of the effect of feeding sulfanilamide to the laying fowl. These workers concluded that sulfanilamide appears to exert a specific inhibition on the secretory ability of the shell glands.

About the same time, Benesch *et al.*,[3] feeding sulfanilamide and sulfapyridine to laying hens, reported that carbonic anhydrase appeared essential for shell formation. Their working hypothesis was based on the following: (a) the suggestion of Meldrum and Roughton[4] that carbonic anhydrase might play a rôle in egg-shell formation; (b) the finding by Common[5] that the carbonic anhydrase activity of the uterine epithelium is higher than that of the remaining oviducal tissues, (c) the demonstration by Keilin and Mann[6] that sulfonilamides of the $R\text{-}SO_2NH_2$ type specifically inhibit carbonic anhydrase. This property, however, is absent in all compounds in which the sulfonamide group is substituted, as it is, for example, in sulfapyridine and sulfathiazole. Benesch *et al.*[3] fed single doses varying from 0.1 gm/kg to 0.2 gm/kg of sulfanilamide to laying hens and observed very thinned and pitted shells. The same birds were afterwards given a similar dose of sulfapyridine, and the shells obtained were normal throughout.

The writers undertook experiments with several sulfonamides of the unsubstituted and substituted type. The sulfonamides were fed at levels of 0.3 or 0.5 per cent. of the dry mash. The experimental period lasted one week and was preceded and followed by one-week control periods.

It was found that unsubstituted sulfonamides such as Soluseptazine and Neoprontosil caused thin rough-shelled eggs similar to those obtained upon feeding sulfanilamide. The substituted sulfonamides such as sulfathiazole, sulfaguanidine, sulfamerazine and sulfadiazine had a negligible effect on shell thickness. The shells were smooth, though they appeared slightly pitted when observed under low magnification.

These results so far confirmed the conclusions of Benesch *et al.*[3] On the other hand, repeated experiments with sulfapyridine consistently revealed a decrease in shell thickness as well as a rough surface, whether this compound was fed for several days or in a single dose. The data are presented in Table 1 and Fig. 1.

TABLE 1

EFFECT OF SULFAPYRIDINE ON SHELL THICKNESS

Per cent. sulfapyridine in dry mash	Bird number	Mean shell thickness (mm)		Per cent. decrease in shell thickness
		Control period	Experimental period	
0.3	1	0.343 ± 0.004*	0.275 ± 0.013	19.7
	2	0.354 ± 0.004	0.265 ± 0.006	25.0
	3	0.371 ± 0.004	0.283 ± 0.012	23.7
	4	0.354 ± 0.004	0.267 ± 0.016	24.4

*P. E. of the mean.

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