lar atrophy but no gynecomastia.

No consistent pattern of gonadotropic or other pituitary hormone storage was found in the cirrhotic men that could be correlated with the presence of gynecomastia, hepatoma or other malignant tumors.

Discussion. Within the acknowledged limitations of the variable human material available, the qualitative type of pituitary bioassay employed, and the complicated endocrine changes that may accompany cirrhosis, the observation of ample stored pituitary gonadotropin and low or absent urinary gonadotropin excretion is enlightening. Usually urinary gonadotropin excretion parallels the hypophyseal storage of gonadotropin, but in human cirrhosis there is a discrepancy. Also, in experimental starvation a dissociation of the hypophyseal gonadotropin content and circulating hormone has been reported(6).

The low pituitary contents of gonadotropin in 2 of the cirrhotics who died of hemorrhage and shock are unexplained, unless unusual stress had been associated with a rapid release of stored hormone. In the other man with low pituitary gonadotropin, Stilbestrol therapy might be implicated since several other patients treated with large doses of estrogens have had very low levels of pituitary gonadotropin storage(7). Malnourished humans have also showed both low levels of pituitary gonadotropin storage and urinary excretion(8,9).

Without excluding the possibility that in cirrhosis pituitary gonadotropins might be released and then inactivated before their urinary excretion, an explanation of these findings is proposed. In cirrhosis the moderately increased levels of circulating estrogen in men appear to block the secretion of pituitary gonadotropin, with a consequent reduction of the urinary excretion levels. In some other conditions, such as women with polycystic ovaries, the same apparent dissociation may occur between pituitary basophil hyperplasia and low or normal urinary gonadotropin levels(10,11).

Summary. Bioassays of gonadotropin from 13 cirrhotic men showed ample hypophyseal gonadotropic hormone in 10. Urinary gonadotropin excretion was not detected in 5 of 9 cirrhotics, and had low normal values in the other 4. Pituitary gonadotropin release may be inhibited in some cases of cirrhosis, possibly by estrogen.

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Cross Reactions with Tuberculins on Guinea Pigs with Single and Double Experimental Infections with Mycobacteria.* (29439)

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For many years, tuberculosis-like infections in man produced by mycobacteria other than *Mycobacterium tuberculosis* have been reported in the literature(1-3). In most cases these reports, consisted of descriptions of single cases or very small groups of cases, with

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very little relevance to interpretation of epidemiologic surveys with skin tests or actual diagnosis of clinical tuberculosis. In 1958 however, Edwards and Palmer published preliminary results of their work with purified protein derivatives (PPD's) prepared from "atypical" acid-fast organisms(4). From this and subsequent work(5) they concluded that large numbers of people in the general population were being infected with mycobacteria other than the tubercle bacillus, and that these organisms were producing cross sensitivity to human tuberculin. In addition their studies of experimental infections with these organisms in guinea pigs(6) revealed that these animals often showed cross sensitivity to heterologous mycobacterial antigens, but that the purified protein derivative homologous to the sensitizing organism would always give the largest skin reaction.

We skin tested 164 medical students and 80 student nurses at Duke University with 5 TU doses of PPD's prepared from the human and other types of mycobacteria. Among the medical students, 14% reacted to the human PPD, 14% to the photochromogen PPD, 23% to the Battey PPD, and 51% to the scotochromogen PPD. The same trend was noted in the student nurses, although the total number of reactors was about half of the medical student group(7).

In this paper are reported the patterns of cross reactivity produced in guinea pigs that have been sensitized with one or two strains of human and unclassified mycobacteria and subsequently skin tested with homologous purified protein derivatives prepared from these mycobacteria.

Materials and methods. Five strains of mycobacteria were chosen for this study. They were a "Battey," *Mycobacterium fortuitum, Mycobacterium tuberculosis* (R1v), *Mycobacterium kansasii* ("Yellow" bacillus, a photochromogen), and a scotochromogen. The standard R1v culture was used for the human bacillus. The *Mycobacterium kansasii* was isolated by Stenken from a laboratory specimen. The Battey-Boone strain, the *Mycobacterium fortuitum,* and the scotochromogen (343 Bridge) were supplied to us by Dr. Lydia Edwards. These were the strains of Battey, fortuitum, and scotochromogen from which the corresponding PPD's were prepared.

The Battey, fortuitum, photochromogen, and scotochromogen were grown on Petragnani media at 37°C for 4 to 6 weeks. A sufficient quantity of bacilli was removed from each culture and weighed to produce a final concentration of 4 mg/cc (moist weight) when suspended in 0.85% saline. Equal dispersion of the bacilli in saline was carried out by the use of a Potter-Elvehjem tissue homogenizer. The resulting bacterial suspensions in saline were used for guinea pig inoculation. The R1v was suspended in saline to a final concentration of 1 mg/cc saline (moist weight).

We had learned by previous experience that a high level of hypersensitivity to 5 TU doses of purified protein derivatives could be produced in guinea pigs by using very large male animals and by inoculating them intratesticularly and intrainguinally with large numbers of living bacilli. Smaller guinea pigs reacted poorly to the 5 TU doses and frequently died before completion of the experiment. For these reasons male albino guinea pigs weighing in excess of 1,000 g were used almost exclusively. They were divided into 5 experimental groups of 20 to 40 animals per group. Group I animals received a single dose of 4 mg Battey bacilli suspended in 1 cc saline divided equally among the 2 inguinal areas and the 2 testes. In an analogous manner Group II animals received 4 mg of fortuitum bacilli, Group III animals 1 mg R1v bacilli, Group IV animals 4 mg photochromogen bacilli, and Group V animals 4 mg scotochromogen bacilli.

Two months after sensitization each guinea pig was skin tested with 5 TU doses of standardized human PPD, avian PPD, and other purified protein derivatives prepared from the previously mentioned unclassified strains and furnished by the Dept. of Health, Education, and Welfare. Each test was given on the clipped side of the guinea pig as an intradermal injection of 0.1 cc PPD antigen. In all guinea pigs each skin test was done in duplicate, and in many cases in triplicate, in order to check for technical error. The skin reac-

TABLE I. Summary of Tuberculin Reactions in Guinea Pigs with Single and Double Infections.

Animal group	Sensitizing organisms	No. of groups of tests performed	Skin reactions (mean mm induration at 48 hr)					
			PPD-A*	PPD-B*	PPD-F*	PPD-S*	PPD-Y*	PPD-scot.*
I	Battey	68	6.4	8.2	.7	1.5	.9	5.6
II	Fortuitum	48	—	4.3	16.4	2.6	1.6	—
III	R1v	110	4.6	3.6	.8	13.1	2.8	3.9
IV	Photochromogen	56	3.7	3.3	—	4.2	9.3	3.3
V	Scotochromogen	49	5.8	4.4	—	1.4	.4	12.2
VI	R1v-Battey	14	—	11.3	1.1	14.9	3.9	13.4
VII	Battey-R1v	16	12.1	13.1	2.4	14.4	3.9	10.4
VIII	Photochromogen-R1v	18	—	4.0	.6	12.6	14.0	—
IX	R1v-Scotochromogen	29	—	9.1	2.8	15.7	3.2	18.8
X	Scotochromogen-R1v	10	6.8	9.2	1.8	12.7	2.9	13.2

* PPD-A = avian PPD; PPD-B = Battey PPD; PPD-F = fortuitum PPD; PPD-S = human PPD; PPD-Y = photochromogen PPD; PPD-scot. = scotochromogen PPD.

tions were read at 48 hours to the nearest millimeter and recorded as millimeters of induration. Erythema was ignored. In all instances one of the authors injected the skin test antigen and the other measured the reactions. In the duplicate and triplicate skin tests performed with the same antigen, a difference of 3 mm or less between 2 reactions was considered an identical reaction. Any difference greater than 3 mm was interpreted as technical error. This error varied from 7% to less than 1% with a mean error of 4%.

To study the possible modifications on the patterns of cross reactivity that might be produced by introduction of a second strain of organism into the sensitized pigs, the following procedure was followed. After completion of the skin testing of the 5 groups of guinea pigs, selected groups were sensitized to a second strain of organism prepared and inoculated in a manner and quantity analogous to the initial sensitizations. The Battey group of animals received R1v bacilli. The fortuitum group of animals was not used. The R1v group was subdivided into 2 smaller groups each one of which received Battey and scotochromogen bacilli respectively. The photochromogen group received R1v, and the scotochromogen group received R1v. These doubly sensitized guinea pigs were skin tested again 2 months later. For all the animal groups subjected to double sensitization, control animal groups with a single infection only were maintained. These groups were skin tested simultaneously with the double infection groups. In all instances their patterns of skin reactivity remained the same as for the patterns in the large experimental groups of animals sensitized to one strain of organism.

Results. In these experiments approximately 2,000 tuberculin tests were performed on 217 guinea pigs. The results of these skin tests on guinea pigs expressed as mean millimeters induration are shown in Table I. In all of these animals with single infections the PPD antigen homologous to the sensitizing organism produced the largest skin reaction. The Battey group of animals exhibited reactions of 8.2 mm to PPD-B, 6.4 mm to PPD-A, and smaller reactions to the other antigens. The fortuitum group of animals showed skin reactions of 16.4 mm to PPD-F, with very low levels of cross reactivity from the other antigens. PPD-scot. was not available at the time this group of animals was tested. The R1v group showed skin reactions of 13.1 mm to PPD-S, with small reactions to the other antigens (Fig. 1). The photochromogen group showed reactions of 9.3 mm to PPD-Y with a moderate degree of crossing from the other antigens (Fig. 2). The scotochromogen group showed reactions of 4.4 mm to PPD-B, and 12.2 mm to PPD-scot. with smaller reactions to the other antigens. PPD-F was not available at the time that the photochromogen and scotochromogen animal groups were skin tested.

Animal groups I, III, VI, and VII in Table I summarize the comparisons between animals in which either Battey or R1v sen-

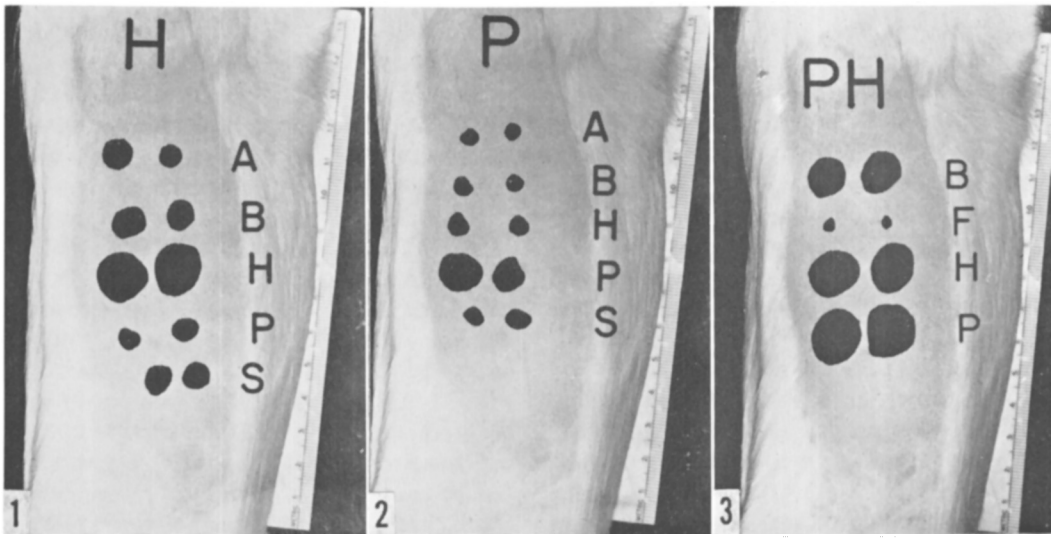

FIG. 1. Duplicate tests on a guinea pig sensitized to a human organism (R1v) and tested with Avian (A), Battey (B), Human (H), Photochrome (P), and Scotochrome (S) PPD. Traced from a Kodachrome slide and reproduced at approximately ⅔ natural size.

FIG. 2. Duplicate test on a guinea pig sensitized to *Myco. kansasii* (Photochrome) and tested with the other PPD's. Traced from a Kodachrome slide and reproduced at approximately ⅔ natural size.

FIG. 3. Duplicate test on a guinea pig sensitized to a human organism (R1v) and tested after 2 months. Resensitized with *Myco. kansasii* (Photochrome) and retested after an additional 2 months. Traced from a Kodachrome slide and reproduced at approximately ⅔ natural size.

sitization was present and animals in which double sensitization with R1v and Battey was present. In the presence of this double sensitization PPD-S and PPD-B both evoked large skin reactions. In all cases these reactions were greater than the reactivity to the homologous PPD seen in the single infections. In addition a striking increase in cross reactivity to PPD-scot. occurred. Enhancement of the PPD-F and PPD-Y reactions occurred only slightly if at all.

Animal groups III, V, IX, and X summarize the comparisons between animals in which either R1v or scotochromogen sensitization was present and animals in which double infections with R1v and scotochromogen bacilli were induced. In these animals PPD-S and PPD-scot. produced large reactions. In all animals tested these reactions were equal to or greater than the reactivity to the homologous PPD with single infection. In these animals also, skin reactivity to PPD-B more than doubled in size.

In those guinea pigs sensitized to both R1v and photochromogen bacilli, reactions to PPD-S and PPD-Y were large and approximately equal in size, but cross reactivity with other PPD antigens remained essentially unchanged (Fig. 3) from those observed with single infections only (animal groups III, IV, and VIII).

Discussion. The results of our skin testing of medical students and nurses at Duke University(7) have confirmed the hypothesis that subclinical infections with unclassified mycobacteria occur in the general population. These data have suggested further that both cross reactions and multiple infections may be present in these students. The animal experiments reported here were designed to show whether or not single and multiple infections with human and unclassified mycobacteria could be specifically diagnosed on the basis of quantitative skin testing with purified protein derivatives in 5 TU doses. The previous studies of Edwards *et al*(6) and Magnusson(8) have shown that in guinea pigs infected with one strain of mycobacteria, the PPD homologous to the sensitizing organism gives the largest skin reaction in the great majority of organisms studied. Their work has further shown the very

close antigenic relationship between avian and Battey strains. Our results in singly infected animals confirm these findings. For the fortuitum, R1v, photochromogen, and scotochromogen animal groups, the homologous PPD produced a skin reaction that was a minimum of 6 mm greater than the reactions produced by the other antigens. We would conclude from these observations that when a single infection with one of the above named organisms is present, it can be detected and separated from the other possibilities. Infection with the Battey bacillus offers more difficulty. Not only is it closely related antigenically to avian strains but also only slightly less so to the scotochromogen. This is illustrated in our Battey group of animals in which their skin reactivity to PPD-scot. averaged only 1.6 mm less than their reactivity to the homologous PPD-B.

With double infections 2 different patterns of reaction could be detected. In the photochromogen-R1v animal group, PPD-Y and PPD-S produced large, almost identical reactions, while the reaction to PPD-B and PPD-F remained quite small. For this combination then, skin testing with the proper antigens would reveal the presence of a double infection. In the Battey-R1v combination, however, the marked enhancement of the PPD-scot. reaction in addition to the large PPD-B and PPD-S reactions produces a pattern of skin tests suggesting the presence of not 2 but 3 infections. To a slightly less marked degree, the same pattern is produced in the R1v-scotochromogen combination. The mechanism of this phenomenon is not completely clear but is probably the result of reinforcement of the skin reactions to the antigens common to Battey and the scotochromogen by the antigens contained in the R1v bacillus also common to the Battey and scotochromogen organisms.

Summary. Primary sensitization of guinea pigs with Battey, fortuitum, human, photochromogen, and scotochromogen mycobacteria produced large tuberculin reactions to the homologous PPD's but less and varying degrees of cross reactivity with other PPD's. Double infections in guinea pigs from human and scotochoromogen bacilli gave large cross reactions with PPD-A and PPD-B, but not with PPD-Y or PPD-F. Double infections with human and photochromogen bacilli gave large reactions to PPD-S and PPD-Y but not to PPD-B or PPD-F. Double infections with Battey and human bacilli gave large cross reactions to PPD-scot. but not to PPD-Y or PPD-F. It is concluded from these observations that skin testing with a battery of PPD's at the 5 TU dose level may be a valuable diagnostic tool in the presence of infection by a single organism. This value decreases in the presence of a double infection.

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