TABLE II. Weight Gain of Plateaued Adult Female Rats Treated with Pituitary Growth Hormone and Stanolone, in Successive 10 Day Intervals before and during Treatment.

Treatment and dose	No. of rats	Wt gain (g) 10 days pretreat.	10 day intervals of treatment 1st	2nd	3rd	4th	5th
Growth hormone, 1 mg/rat/day	8	0.9 ± 2.0*	38.6 ± 2.8	21.8 ± 4.6	−5.8 ± 5.7	−3.0 ± 3.1	−1.6 ± 1.0
Growth hormone, controls	20	0.7 ± 0.7	4.9 ± 1.3	2.8 ± 1.1	−1.2 ± 1.3	2.9 ± 1.1	−2.4 ± 1.0
Stanolone, 5 mg/rat/day	6	1.2 ± 1.6	19.3 ± 2.3	6.3 ± 1.3	6.0 ± 0.5	3.7 ± 1.6	
Stanolone, controls	18	3.1 ± 0.8	2.3 ± 0.7	0.0 ± 1.4	5.1 ± 1.1	2.0 ± 1.2	

* Mean ± stand. error in all cases.

mone and stanolone were given to plateaued adult female rats of the same strain. These hormones, at dose levels capable of giving a major growth response during the first 20 days of treatment, gave little response after the first 20 days.

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Serum Protein-Bound Carbohydrates and Lipids in Experimental Tuberculosis. (22826)

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Guinea pigs inoculated with tubercle bacilli reveal characteristic serum protein changes which resemble to a great degree those in human tuberculosis; serum gamma globulin is elevated and albumin reduced(1,2). These serum protein changes, especially the gamma globulin elevation, have been related to chronic inflammation and hypersensitivity reactions. However, similar changes occur in chronic hepatic disease. It appeared therefore desirable to further characterize the serum protein changes produced by chronic tuberculosis.

A rise of serum mucoproteins (which are alpha globulins)(3) in experimental tuberculosis(4), in human tuberculosis(5) and in experimental brucellosis(6) has been demonstrated. This rise is absent in hepatic injury (7). Since methods have become available recently to demonstrate serum protein-bound lipids(8) and carbohydrates(9) by zone electrophoresis it appeared worthwhile to study their levels in experimental tuberculosis. To accentuate the serum protein changes, animals with long standing tuberculosis were investigated. The levels were compared not only with those of healthy control guinea pigs but also with animals in which treatment with

isoniazid temporarily prevented development of tuberculosis(10).

Materials and methods. Serum was obtained from 12 guinea pigs inoculated subcutaneously into the right inguinal region with 0.1 mg of tubercle bacilli, strain H37Rv, and exhibiting far advanced tuberculosis at autopsy. Another group of 7 animals similarly infected received 20 mg isoniazid daily for 2 months following inoculation and were free of tuberculosis grossly and microscopically at autopsy. All animals were sacrificed 8 to 16 months after inoculation simultaneously with 12 control guinea pigs kept for the same period and free of any tuberculosis at autopsy. The following were determined in the serum: total proteins by micro-Kjeldahl and Nesslerization; gamma globulin turbidimetrically according to Huerga and Popper (11); mucoproteins also turbidimetrically according to Huerga *et al.*(12); hexosamines according to Elson and Morgan(13); protein-bound hexoses according to Lustig and Langer(14) and total lipids turbidimetrically according to Huerga *et al.*(15). In addition 3 paper strips, on which serum had been placed, were exposed to vertical zone electrophoresis. One was subsequently stained with bromphenol blue for proteins(16), another with periodic acid leucofuchsin (Schiff reaction) for protein-bound carbohydrates(9) and a third one with Oil Red O, for protein-bound lipids according to Durrum(8). The relative amount of protein as well as protein-bound carbohydrates and lipids were determined by integrating photometric scanner (Analytrol). The amounts found were expressed in percentage, and absolute amounts were calculated using as reference for proteins the total protein; for protein-bound carbohydrates the sum of hexosamines and protein-bound hexoses; and for protein-bound lipids the total lipids. In addition lipid and carbohydrate content of each serum protein fraction was calculated and expressed in percent of that fraction.

Results. In guinea pigs with active tuberculosis serum albumin was moderately reduced and gamma globulin and mucoprotein were significantly elevated. These changes, as well as those to be reported below, were absent in inoculated animals in which isoniazid treatment prevented active tuberculosis.

Total serum lipids as well as lipoprotein fractions were not influenced by tuberculosis. Both total serum hexose and hexosamine were slightly elevated in active tuberculosis as were all protein-bound carbohydrates, if expressed in absolute amounts. However this increase was not statistically significant and the relative distribution of carbohydrates was not significantly altered. Carbohydrate concentration of gamma globulin however, was distinctly depressed in guinea pigs with tuberculosis while that of alpha globulin and beta globulin were not significantly altered.

The very small amount of carbohydrates in the large fraction of albumin discouraged calculation of this figure.

The failure of the carbohydrate moiety of the gamma globulin to rise parallel with protein, resulted in a lower carbohydrate concentration in this protein fraction. This reflected itself in the ratio of carbohydrate concentrations, (carbohydrate percent in alpha globulin/carbohydrate percent in gamma globulin) and in the alpha globulin/gamma globulin ratio of the proteins (Table I). The former increased, while the latter decreased in tuberculous animals.

There is an increase in mucoproteins in tuberculous animals.

Discussion. Since the most significant alteration in protein fractions in experimental guinea pig tuberculosis involves albumin and gamma globulin, it is not surprising that lipoproteins, mainly components of alpha and beta globulins, are not significantly altered in these conditions. The slight rise of alpha globulin under these circumstances is reflected in marked elevation of mucoproteins which are alpha globulins(3). Since mucoproteins represent only a very small fraction of alpha globulin their marked rise which also runs parallel with elevation of total carbohydrates, is not sufficient to produce significant changes of serum concentration of the alpha globulin. The relatively small rise is associated with a parallel rise of alpha globulin-bound carbohydrate which suggests that the excess amount of alpha globulin does not differ in carbohydrate content. However, this rela-

TABLE I. Serum Protein Fractions in Control and Tuberculous Guinea Pigs.

	Controls		Tuberculous active			Tuberculous successfully treated		
					OD			OD
	Mean	SEm	Mean	SEm	SE-diff.	Mean	SEm	SE-diff.
No. of animals	14		12			7		
Albumin, g %	3.1	.13	2.6	.03	3.6	3.1	.10	.0
α-globulin, "	1.6	.13	1.9	.04	2.1	1.7	.04	.7
β-globulin, "	.9	.04	.8	.05	1.5	.8	.06	.0
γ-globulin, "	.6	.05	1.6	.07	40.0	.8	.10	1.8
Mucoproteins, mg %	323	21	537	24	6.5	346	15	.9
Total lipids, "	217	6.2	201	16	.9	191	17	1.4
α-lipoproteins, "	128	7.0	131	17.7	.1	130	17	.1
β-lipoproteins, "	69	7.0	64	8.0	.5	57	6.4	1.2
Protein-bound hexose, "	110	9.4	119	5.0	.8	103	44	.7
Hexosamine, "	174	9.0	194	6.6	1.9	166	3.5	.8
Protein-bound CHO, "	296	12.3	312	8.6	1.1	269	6.3	1.2
Albumin-bound CHO, "	18	4.0	16	3.5	.4	13	3.5	.9
α-globulin-bound CHO, "	205	7.5	222	7.7	1.6	191	5.3	1.5
β-globulin-bound CHO, "	43	3.2	42	3.0	.2	35	2.0	2.2
γ-globulin-bound CHO, "	22	1.6	33	2.6	3.6	27	2.0	2.0
CHO, % in α-globulin	12.3	.6	11.3	1.0	.8	11.3	.8	1.0
" , " " β-globulin	4.6	.4	5.2	.3	1.2	4.4	.5	.3
" , " " γ-globulin	4.8	.6	2.3	.2	4.0	4.7	.3	.2
α-globulin/γ-globulin	3.5	.3	1.2	.1	20.0	2.5	.3	2.3
CHO, % in α-globulin / CHO, % in γ-globulin	3.4	.4	6.3	.9	9.4	2.9	.5	2.2

SEm is stand. error of the stand. dev.

$\frac{\text{OD}}{\text{SE-diff.}}$ is the observed difference between control and exp. group divided by stand. error of that difference (above 2.5 significant).

tively small increase prevents a more definite conclusion. Similarly the constancy of the beta globulin level and the small amounts of carbohydrate-bound albumin do not favor any calculation.

However, the contrast between the marked rise of serum gamma globulin with only a small elevation of its carbohydrate moiety, best reflected in the difference between the alpha globulin/gamma globulin ratio of protein and the ratio of the carbohydrate concentration of these proteins, permits the conclusion that the excess gamma globulin formed in tuberculosis is poor in carbohydrates. If calculations of the carbohydrate concentration of the excess gamma globulin in the tuberculous animals are permissible from the averages, this increment in the amount of 1 g per 100 ml has a carbohydrate moiety of almost 1% in contrast to the gamma globulin in control guinea pigs with a mean carbohydrate concentration of 4.8%. This is in contrast, for instance, to the elevated carbohydrate concentration of the excess gamma globulin in myeloma(17,18) and the unchanged concentration in infectious mononucleosis(19), experimental brucellosis(6) and hepatitis(20), and the small differences recently demonstrated by Müller-Eberhard and Kunkel(21) in the carbohydrate concentration in various components of gamma globulin in normal and abnormal circumstances. The findings in experimental tuberculosis suggest that the excess gamma globulin represents a reaction globulin(22) different in nature from the normal gamma globulin and possibly formed in other sites.

Summary. The previously demonstrated reduction of serum albumin and elevation of gamma globulin and mucoprotein in experimental guinea pig tuberculosis is associated with a slight elevation of total serum hexose

which is to the greatest extent accounted for by a slight rise of the carbohydrate containing alpha globulin. No evidence was obtained that the carbohydrate content of this small increment differs from that of the normal alpha globulin. In contrast the marked rise of gamma globulin is associated with an only very small increase of gamma globulin associated carbohydrates. This suggests that the excess gamma globulin in experimental tuberculosis differs from the normal gamma globulin by a very low carbohydrate content and suggests a different nature and possibly a different origin of this increment. The serum protein-bound lipids are not altered in experimental tuberculosis.

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Increased Metabolism in Fat-Deficiency: Relation to Dietary Fat.* (22827)

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Elevation of metabolic rate is consistently observed in rats fed a diet devoid of fat. As early as 1931, Wesson and Burr made reference to increased basal and carbohydrate assimilatory rates(1), findings which were confirmed and extended by Burr and Beber(2). It has been pointed out that disturbance in energy balance as indicated by increase in basal oxygen consumption is probably the earliest and most fundamental manifestation of the fat-deficiency syndrome, being recorded within 7-14 days after the start of the experimental diet in rats(3,4).

The manifestations of fat-deficiency are corrected by the administration of linoleic acid in amounts too small to exert an influence as a metabolite (*i.e.* "fat as fat *per se*"), indicating the specific role of this fatty acid as an essential substance(5). It is the purpose of this presentation to report the effect on oxygen consumption and body weight of

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