tivity in a modified hot plate method and acute toxicity were determined in mice. The data obtained from the analgetic test were statistically analyzed and the relative potency of the analogs in relation to morphine sulfate was determined. The structure-activity relationships of the analogs with respect to relative analgetic potency and acute toxicity have been discussed.

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Electrophoretic and Immunolelectrophoretic Studies of the Serum of Germfree and Conventional Guinea Pigs.* (29407)

GEORGE B. OLSON AND BERNARD S. WOSTMANN (Introduced by Morris Pollard)
Lobund Laboratory, University of Notre Dame, Notre Dame, Ind.

When germfree guinea pigs were kept on a diet of essentially vegetable origin, one year old animals displayed low serum beta globulin levels and gamma globulins were undetectable(1). Using a modification of the same diet Newton and DeWitt showed that, although the caesarian born germfree guinea pig starts life with a gamma globulin level of approximately 7% of total serum protein, at the age of 3 months this level has already declined to values of 2 to 3%(2). Incorporation of bovine milk proteins into the diet led to the appearance of appreciable amounts of gamma globulins and an increase in beta globulin levels of the serum of the mature germfree guinea pig(3).

We present here an analysis of the sera of germfree and conventional guinea pigs of various ages. The results indicate that both microbial and dietary stimulation play an important role in determining the quantitative pattern of the serum globulins.

Materials and methods. Animals. The germfree and conventional guinea pigs used were obtained from the Lobund animal colonies. The first group of 4 germfree and 6 conventional guinea pigs had been fed the L-445 diet(4). Subsequently, a second group of 18 germfree and 13 conventional guinea pigs, of varying ages, were maintained on diet L-462, a formula high in bovine milk protein(5). Animals were bled via cardiac puncture.

Quantitative electrophoresis. Electrophoretic patterns of the sera from the first group were obtained with the Antweiler Micro-free boundary electrophoresis apparatus, using a sodium barbital buffer of pH 8.6 and the ionic strength of 0.12(1). Analysis of sera from the second group was made on cellulose acetate paper with the Shandon Universal Electrophoresis apparatus, and a sodium barbital buffer of pH 8.6 and ionic strength of 0.06. Cellulose strips were stained with Amido Black 10B(6) and analyzed in a light reflecting densitometer (Chromoscan by Joyce Loeb & Co. Ltd.).

Antisera. Three different anti-guinea pig-rabbit immune sera were produced using 3 different lots of conventional guinea pig serum. Each serum lot consisted of the sera from 2 different animals. Rabbits were subjected to the following schedule: 40 mg guinea pig serum protein, 0.25 ml of Freund's

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Complete Adjuvant and 0.5 ml mineral oil was injected intraperitoneally (i.p.), followed on 2 subsequent weeks by single i.p. injections of 40 mg guinea pig protein serum. On the sixth week rabbits were tested for Arthus reaction, given a challenging dose of 70 mg serum protein i.p. and bled via cardiac puncture 7 days later.

Immunoelectrophoresis. Immunoelectrophoresis of all the sera from the second group was performed using a 1% highly purified agar gel and a sodium barbital: HCl buffer of pH 8.2 and ionic strength of 0.10(7). Immunoelectrophoretic patterns (IEP) were photographed after washing and staining with Amido Black 10B.

Results. Data on total serum protein concentration, per cent composition and protein mg per cent composition are presented in Table I. The results from animals fed diet L-462 are subdivided into age groups of 7 days, 2-4 months, 8 months and over one year to show serum protein fluctuation with age in germfree and conventional guinea pigs.

Levels of total protein in the guinea pigs fed L-462 diet are constant at approximately 5.5 to 6.0 g per cent except for the 4.86 and 4.79 g/100 ml found in the 7 day old and the 2-4 months old germfree animals. The year old germfree and conventional guinea pigs fed diet L-445 (of vegetable origin) display approximately 0.8-0.9 g per cent less total protein than their counterparts fed the L-462 diet (containing bovine milk proteins).

The serum of conventional one year old guinea pigs on diet L-445 contains 63.8% albumin, 23.1% alpha, 8% beta, and 5.1% gamma globulin as depicted by moving

TABLE I. Serum Protein Fractions of Germfree and Conventional Guinea Pigs.

Type and age	Total protein, g/100 ml		Albumin	1	2	3 α	4 β	5 γ	Double gamma globulin lines*
Diet L-462†									
Conv. 7 day (3)	4.86 ±.3	Comp. %	57.4	2.0	5.0	20.6	7.2	6.6	3/3
		mg %	2582 ±119‡	131 ±38	228 ±24	1089 ±143	328 ±48	297 ±48	
Germfree 2-4 mo (5)	4.79 ±.24	Comp. %	62.3	2.9	6.0	21.3	5.3	1.9	1/6
		mg %	3025 ±218	138 ±29	281 ±41	989 ±45	261 ±69	94 ±16	
Germfree 8 mo (4)	5.59 ±.28	Comp. %	58.7	2.5	5.6	20.6	7.7	4.2	4/4
		mg %	3111 ±239	142 ±19	321 ±61	1153 ±86	429 ±47	239 ±49	
Germfree 1 yr (6)	5.77 ±.20	Comp. %	52.7	2.4	6.0	23.9	10.0	5.9	4/5
		mg %	2993 ±211	140 ±21	341 ±9	1379 ±139	568 ±45	345 ±77	
Conv. 2-4 mo (6)	5.60 ±.29	Comp. %	52.0	2.6	5.7	26.2	8.6	5.5	4/5
		mg %	2905 ±153	146 ±18	323 ±43	1452 ±69	490 ±78	309 ±27	
Conv. 1 yr (7)	5.97 ±.22	Comp. %	52.3	2.4	5.2	23.9	9.1	6.5	7/8
		mg %	3111 ±75	143 ±24	310 ±25	1440 ±84	545 ±38	407 ±46	
Diet L-445									
Germfree 1 yr (4)	4.80 ±.19	Comp. %	75.6			23.5	.8	0	
		mg %	3627 ±165			1122 ±97	41 ±7.4	0	
Conv. 1 yr (6)	5.05 ±.11	Comp. %	63.8			23.1	8.0	5.1	
		mg %	3222 ±69			1168 ±53	389 ±37	263 ±58	

* No. of sera in which double gamma was observed/No. of sera tested.
† Diet L-462, electrophoretic determinations made by cellulose acetate zone electrophoresis. Diet L-445, electrophoretic determinations made by Antweiler free boundary method.
‡ SDM (standard deviation of mean).

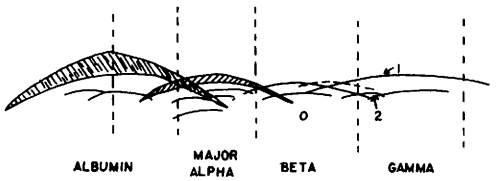

FIG. 1. Schematic drawing of immunoelectrophoretic pattern of guinea pig serum. Precipitin line 1 equals 7S gamma globulin and line 2 equals beta-2M.

boundary electrophoresis. Electrophoresis on cellulose acetate paper resolves the albumin fraction from the sera of similar animals, but raised on diet L-462, into fractions of 52.3%, 2.4%, and 5.2%. The sum of these is 59.9%, which agrees reasonably well with the value of 63.8% obtained in the other group with the Antweiler apparatus. The globulin fractions (fractions 3, 4, and 5 equal alpha, beta and gamma globulin respectively), show excellent agreement by both methods, and it is assumed that the additional components resolved from the albumin on cellulose acetate are a consequence of the greater resolving power of this method and the lower ionic strength employed(8), rather than the difference in the diets on which the 2 groups had been raised.

Table I shows that in the animals fed diet L-462 the main albumin remains constant in all groups (about 3000 mg%), with the exception of the 2582 mg% of 7 day old animals. Globulin fraction 1 is constant in all groups, as is globulin fraction 2 except in the case of the 7 day old group. Globulin 3 (alpha) shows an increase from approximately 1000 mg% in the 7 day old animals to the 1400 mg% level of the year old guinea pigs. This increase in globulin 3 occurs earlier in the conventional animals reaching "adult" levels by 2-4 months of age. Globulin fraction 4 (beta) displays a response similar to that of the alpha globulin in both germfree and conventional animals. At 7 days of age the guinea pig possesses approximately 300 mg% globulin fraction 5 (gamma) which in the conventional animal depicts no change at the 2-4 months age. However, in the germfree guinea pigs at this age there is a decided drop to 100 mg%, a level which then slowly increases to 345 mg% gamma globulin only after the animals are one year of age. In contrast to these data, the year-old animals fed diet L-445 showed significant differences with the germfree showing little beta and no demonstrable gamma globulin.

No difference could be detected between the IEP of adult germfree and conventional guinea pigs reared on diet L-462. A schematic drawing of the typical IEP is depicted in Fig. 1 and shows the mean number of precipitin lines for the albumin, alpha, beta and gamma globulin regions as 3, 5, 3, and 3 respectively. These regions are characterized by the electrophoretic mobilities of agar gel electrophoretic conditions and do not depict the greater resolution of the albumin range obtained with cellulose acetate electrophoresis. Fig. 1 also shows the presence of a low mobility double gamma globulin system consisting of normal 7S gamma globulin and a second apparently more homogenous globulin of low electrophoretic mobility. Fig. 2 shows the IEP for germfree and conventional guinea pigs of various age groups and indicates that the low mobility double gamma globulin system is present in all groups except the 2-4 month old germfree guinea pigs (Fig. 2b). Table I presents the number of sera in which this double gamma system

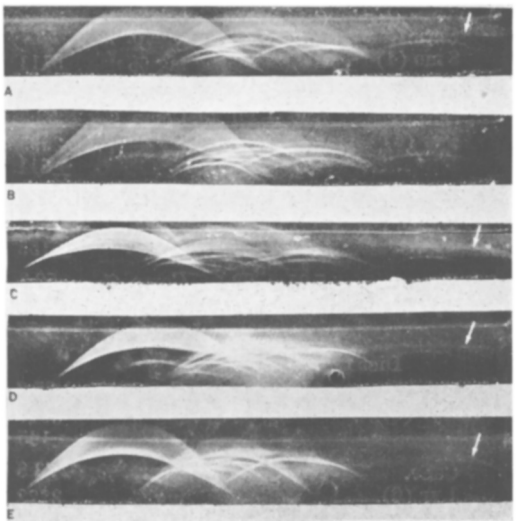

FIG. 2. Immunoelectrophoretic patterns of (a) 7 day old guinea pigs, (b) 2-4 month old germ-free guinea pig, (c) adult year-old guinea pig, (d) 2-4 month old conventional guinea pig, (e) adult year-old conventional guinea pig.

was observed over the number of sera tested for all age groups. The arcs of these 2 gamma components possess entirely different symmetry, and their presence has been demonstrated with 3 different antisera at antiserum/antigen volumes of 30/1 to 100/1. This eliminates the possibility that they may be due to antibody/antigen artifacts or due to Liesegang lines(9). Furthermore, the position of this second gamma line is such that it is not comparable to the beta-2M of the guinea pig (Sell, personal communication) nor to the beta-2A of the human(7). However, Fig. 1 does depict one precipitin line (dashed line in beta zone) which appears in approximately 40% of the IEP, and by reference seems to be comparable to either the beta-2A of other species or possibly to one of the 7S gamma globulins observed by Benacerraf(10).

Discussion. The feeding of steam sterilized diets L-445 and L-462 to the germfree and conventional guinea pigs apparently does not constitute a severe nutritional stress. Total serum protein values for the conventional pigs are comparable with those previously reported(11). Germfree animals show values similar to their conventional counterparts, except for the 2-4 month old group (diet L-462) in which the reduction in serum globulins is obviously associated with a low level immune response. An early reduction in gamma globulin levels of 3-week-old conventional and germfree pigs is suggested in the data of Newton and DeWitt(2). This is presumably due to dilution and catabolism of the placentally transferred gamma globulins. Data obtained in our laboratory showed the same tendency(3). The present data do not show this phenomenon, because most of these conventional animals were older and already contained metabolic systems in the process of synthetizing globulins in response to a variety of antigenic stimulations. In the absence of a viable microflora the germfree guinea pig maintains this reduced gamma globulin level for a longer period. However, when fed diet L-462, the gamma globulin levels eventually increase until at the age of one year values are not significantly lower than those found in conventional animals. The IEP shows the 7S gamma globulin line to extend well into the beta globulin range. Therefore, some of the changes in the gamma globulin range will also be reflected in the concentrations measured in the beta globulin range (Fig. 1). A true increase of beta globulins with age is suggested. This effect appears to depend in part on the presence of an intestinal microflora, but is also influenced by the nature of the (sterilized) diet. The feeding of the complex vegetable and animal protein diet (L-462) leads to a considerable increase in this protein fraction, even in the germfree pigs.

The alpha globulin fraction of guinea pig serum consists of one major and 4 to 5 minor components (Fig. 1). Comparable levels of alpha globulin are found in one-year-old germfree and conventional guinea pigs. Both diets seem to give rise to levels of the same order of magnitude. However, the diet L-462 data shows a retarded increase from neonatal to "adult" levels in the germfree animal, suggesting that at least one of the components of this fraction is dependent on or related to antigenic stimulation. In the absence of stimulation by viable microbes, the remaining but less potent antigenic stimuli are dietary components, killed microbial forms, bedding material, and contact with other animals.

It would seem, therefore, that in the guinea pig the major serum globulins all possess one or more protein fractions which are influenced directly or indirectly by antigenic stimulation. Gagi and Pressman have indicated that in this animal, antibodies against insulin occur in both beta and gamma globulin fractions(12).

Even at the time of the lowest gamma globulin concentration a residuum of approximately 100 mg% of protein is present. At the same time the typical 7S gamma globulin line is absent in most of the IEP, leaving only the second precipitin arc in the gamma globulin range (Fig. 2). Previous immunoelectrophoretic analysis of conventional guinea pig sera by Benacerraf *et al*(10) and by Sell (personal communication) have not shown this second precipitin arc. Its position

differs from that of beta-2M (Sell) and from the usual position of beta-2A(7), which has not been described in the guinea pig. Perhaps future work will show this line to represent another member of the known family of immune globulins(13). However, "extra gamma globulins," apparently unaffected by antigenic stimulation, have been found in chicken sera(14). The fact that free boundary electrophoresis showed little or no protein in the gamma globulin range of germfree guinea pigs fed diet L-445 might indicate that on this diet this fraction does not become evident or that it disappears in older animals.

Summary. The serum protein composition of germfree and conventional guinea pigs displays significant differences during young adult life (2-4 months) at which time the alpha, beta, and gamma globulins are less than those of the conventional animal. Immunoelectrophoretic analysis shows one component of the germfree gamma globulin range absent at this time.

In contrast to a diet containing only vegetable protein, which resulted in little beta and no detectable gamma globulin in the serum of adult germfree guinea pigs, a diet composed of complex vegetable and animal proteins stimulated the formation of larger amounts of globulins. This resulted in comparable or not significantly different alpha, beta, and gamma globulin fractions for year old germfree and conventional animals.

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Myocardial Proteins in Dogs of Various Ages.* (29408)

HARRY SOBEL, HERIBERTO THOMAS AND RICHARD MASSERMAN
Veterans Administration Hospital, Sepulveda, and St. Joseph Hospital, Burbank, Calif.

Recently, myocardial infarction was observed in dogs 6-8 years old which were maintained on a diet including thiouracil and cholesterol(1). Myocardial infarction in this species when fed an atherogenic regimen has not been previously reported, presumably because other investigators, obtaining animals at random from shelters, almost always used younger subjects. Accordingly, a systematic study has been undertaken to determine some of the age-dependent variables which could account for the increased susceptibility to myocardial damage(2,3). The present investigation concerns itself with myocardial protein fractions from dogs of different ages. Some age-dependent changes were observed.

Procedure. Male mongrel dogs of known ages were obtained from a reliable supplier.

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