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EFFECTS OF INFECTION ON SELECTED CLINICAL AND BIOCHEMICAL PARAMETERS IN DOGS

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SUMMARY.—Five month old male beagles injected i.p. with a washed 18-hr culture of *Salmonella typhimurium* 1 × 10^9 organisms/kg. responded clinically with a marked depression, anorexia, and a febrile response 12 hr following infection. Animals allowed to live beyond 36 hr showed a decrease in febrile response. Although they still refused food 36 hr following infection, they appeared more alert by 60 hr post-infection. Changes at necropsy were minimal and histologic findings consistent with Salmonella infection were found in both liver and spleen. In animals infected 60 hr liver microabscesses and circumscribed foci of reticuloendothelial cell hyperplasia in the spleen indicated that the animals were responding favourably by mounting a defence against the infection.

As a result of infection there was an outpouring of band neutrophils resulting in an increase in the absolute numbers of circulating white blood cells. Associated with the neutrophilia was an early lymphopenia and eosinopenia.

The total free amino acids were depressed in serum and increased in the liver at all time periods observed, but the degree of change as well as the specific amino acids affected varied; however, alterations in concentration of amino acids in serum of animals infected with Salmonella were similar to those observed in dogs infected with the virus of canine distemper. In both viral and bacterial infected dogs serum phenylalanine was increased and tyrosine remained the same or decreased, resulting in a significant alteration in the phenylalanine/tyrosine (P/T) ratio.

As with distemper infected dogs, an increase of the alpha-2 fraction of serum proteins was detected at 36 hr following infection. There was a decrease in the monosome peak and a corresponding increase in the polysome fraction of the liver post-mitochondrial supernatant preparation associated with infection. The significance of this change in the liver polysome profile following infection is not yet understood.

THE effects of varying levels of caloric intake on the resistance of dogs to the virus of canine distemper were investigated in a series of experiments (Newberne, 1966). Obese dogs had less resistance to the infection and both clinical and biochemical parameters were deranged (Bresnahan and Newberne, 1968; Newberne, Young and Gravlee, 1969).

In a current series of experiments a bacterial agent, *Salm. typhimurium*, was used to infect the dogs. This paper describes the initial study of this series in which the early clinical response and selected biochemical and histopathologic changes were investigated. The dogs were fed an adequate level of calories supplied by a similar diet as in the first series of experiments (Newberne, 1966). In this study major emphasis was placed on the changes in serum free amino acids as a means of

assessing the progress of an infection and to compare observed changes with those following viral infection. Characteristic changes in these parameters might aid in the differentiation between viral and bacterial infection during the incipient stage of disease.

MATERIALS AND METHODS

Four litters of 3 male beagles each, immunized against canine distemper and hepatitis and treated routinely for internal parasites, were obtained at 5 months of age from a commercial supplier.

Upon arrival at our laboratory the dogs were weighed and given a physical examination, identified by ear tattoo, and placed in individual metabolism cages. The lightest and heaviest members of each litter were selected for subsequent infection and the third animal served as a control for the group. They were conditioned for 2 wk prior to infection in a room lighted 16 hr a day (6 a.m.–10 p.m.), with temperature and humidity held constant at 68–72° F. and 40 $\pm$ 5 per cent respectively. Control and experimental animals were housed in opposite ends of the 16 $\times$ 22 ft room.

Feeding was accomplished by offering portions for a 30 min. period at noon of a diet, the proximate analysis of which is shown in Table I; food consumption data is listed in Table II. One litter of 3 dogs was infected a week early on a trial basis and only serum protein results and polysome patterns were obtained.

TABLE I.—*Proximate Analysis of Diet**

	Dry weight (per cent)
Protein	30·0
Carbohydrate	37·0
Fat	24·2
Fibre	1·8
Ash	7·0
	100·0
Calories/100 g.	487·0

* Fed with moisture content of about 70 per cent.

A strain of *Salm. typhimurium* previously described (Young, Chen and Newberne, 1968) was serially passed in 2 mongrel dogs. An isolate in pure culture from the blood of the second dog 72 hr post-infection was used to inoculate trypicase soy broth culture supplemented with 0·5 per cent yeast extract (TSY). The culture was incubated at 37° for 18 hr, collected by centrifugation in sterile capped tubes, and washed 3 times in sterile M/15 pH 7 phosphate buffer. The collected bacteria were suspended in M/15 pH 7 phosphate buffer and 1 ml. amounts were freeze-dried. For infection the reconstituted freeze-dried preparation was passed in 3 18 hr TSY broth cultures, collected by centrifugation, and washed 3 times in M/15 pH 7 phosphate buffer. The final washed suspension was quantitated on a nephelometer and each experimental animal received a calculated dose of 1×10^9 organisms/kg. body weight i.p. contained in 3·2–3·8 ml of buffer at 10 p.m. Control animals received an equivalent amount of sterile phosphate buffer on per kg. basis.

Demise was by administration of 5 ml. of a 5 per cent solution of surital sodium i.v. and exanguation *via* carotid arteries. The abdomen was opened and the liver quickly removed after death. The left lateral lobe of the liver was used for all biochemical determinations and microscopic evaluations were conducted on all lobes. The 3 dogs in each group were killed within a period of 1 hr and the control dog was always the second animal killed. Three dogs were killed at time periods of 12, 36, and 60 hr post-infection.

Pre- and post-infection blood samples were taken between the hours of 8.30 a.m. and 10.30 a.m. Blood for differential cell counts and for the remainder of the haematology studies was taken into EDTA-containing vacuum tubes. Blood for serum analysis was collected from the jugular vein and stored on ice for 2 hr before centrifugation. All serum and plasma samples were clear and free from haemolysis.

Because methodology is so critical to the accurate assessment of amino acid analyses,

our methods will be given in detail. Serum for free amino acid determinations was deproteinized with equal parts of 10 per cent sulphosalicylic acid (SSA). Four ml. of each were mixed and allowed to stand for 10 min. on ice. The mixture was centrifuged (1000 × *g* 5°) for 10 min., the supernatant fluid harvested and placed in a clean centrifuge tube, the precipitate washed twice with 0·1 ml. quantities of 0·1 N HCl, and the washes added to original supernatant. The total volume of supernatant was centrifuged to remove particulate matter and was then transferred to a 10 ml. volumetric flask. The sides of the tube and particulate matter were washed with 1 ml. of 0·1N HCl, centrifuged, and added to the 10 ml. volumetric flask. The sample was made to volume with 0·2N sodium acetate buffer pH 2·2 and then transferred to 4, 3 ml. screw top vials which were frozen −20°. Each ml. of the final dilution contained free amino acids from 0·4 ml. of serum. The samples (2·0 ml., equivalent to 0·8 ml. serum) from individual dogs pre- and post-infection were analysed on columns of custom spherical resin types PA-35 and UR-30 with a Beckman Model 116 Amino Acid Analyser.

Liver samples were prepared by mincing 2 g. of fresh liver in 3 ml. of cold distilled water followed by homogenizing with a Dounce tissue homogenizing unit. It was found necessary to use fresh tissue because with storage at − 20° there is a progressive increase in the level of extractable free amino acids. A 2 ml. sample of homogenate was removed to a capped centrifuge tube and 2·0 ml. of hot 10 per cent trichloroacetic acid (TCA) was added. The mixture was vortexed and centrifuged. Following centrifugation a 2·0 ml. amount of clear supernatant was removed and treated with 2 ml. of a 3/1 chloroform–ethanol mixture. The mixture was agitated by inversion in a conical 15 ml. glass stoppered centrifuge tube to facilitate the removal of lipid and TCA from the supernatant. Following centrifugation the upper aqueous phase, assumed to contain free amino acids from 400 mg. of wet liver (200 mg/ml.), was removed and frozen as for serum. The samples (0·5–1·0 ml, assumed to be equivalent to 100–200 mg. of wet liver) were later analysed in the same manner as described for serum.

Total serum protein was determined by refractometry (Newberne, 1966). Serum fractionation was by means of electrophoresis using horizontal cells and a barbiturate buffer at pH 8·6, 0·10 ionic strength and starting temperature of 5°. Twenty μl. of serum were applied to a 34 cm. strip of Whatman No. 1 Chromatography Paper and a potential of 160 V. was applied for 6 hr. The strips were dried for 15 min. at 100° in a stretched position and then stained with 0·1 per cent Ponceau S red dye in 3 per cent TCA. Following staining, the strips were dried slowly in a warm oven and then scanned in a Beckman analytrol (Beckman Instruments, Palo Alto, California).

Haemoglobulin and packed cell volume, white cell counts and differential counts utilised standard techniques (Schalm, 1965).

Liver polysome profiles were made according to the method of Drysdale and Munro (1968) except that the homogenising solution was 0·375M buffered sucrose and the gradients were linear 15 per cent–50 per cent buffered sucrose.

Tissue samples for histology (Luna, 1968) and for histochemistry were prepared by standard methods (Barka and Anderson, 1965; Wachstein and Meisel, 1956).

RESULTS

The infected dogs were all markedly depressed as early as 12 hr post-infection and remained so through 36 hr. Dogs 11 and 12 failed to eat the food offered, but by 60 hr post-infection they appeared more alert than on the previous day (food intake data shown in Table II).

All infected animals were febrile 10 hr post-infection with an increase in rectal temperature 1·7°–2·3° F. above normal. Animals 8, 9, 11, and 12 had a further increase by 20 hr and in all animals except 9, a further increase was observed by 34 hr post-infection. By 44 hr, dogs 11 and 12 had a slight decrease in temperature and by 58 hr a still further decrease was observed, although the temperature had not returned to a normal value (Fig. 1).

Changes from normal at necropsy were minimal in all dogs. In the infected animals, there was a cloudy ascitic fluid which contained large numbers of polymorph leucocytes, congestion and oedema of mesentery and omentum; lesions were

TABLE II.—*Dietary Intake Pre- and Post-infection**

Dog no.	Time infected (hr)	Food consumed§ Conditioning period Average g./day	Average† g./kg./day	Average cal./kg./day	Infection period Total g./day	g./final 24 hr	Cal./kg./final 24 hr‡
4	0	149	20·9	102	None offered	22·4	109
5	12	149	23·6	115	None offered	24·4	119
6	12	149	20·3	99	None offered	21·9	107
7	0	149	21·8	106	128	18·7	91
8	36	149	20·9	102	104	16·4	80
9	36	149	22·8	111	153	23·6	115
10	0	146	24·4	119	112	0·4	2
11	60	146	22·9	112	153	0·0	0
12	60	148	24·4	119	71	0·9	4·5

* Proximate analysis.

Assuming the figures for calories/g. of 4·1 for protein, 4·1 for carbohydrate, and 9·3 for lipid, 100 g. of dry diet supplies 110 cal. from digestible protein or 23 per cent, 152 cal. from carbohydrate or 31 per cent and 225 cal. from lipid or 46 per cent of the calories.

At 22 g. of dry food per kg. B.W. the diet supplies 6·0 g. of digestible protein, 8·2 g. of carbohydrate and 5·3 g of lipid per kg. B.W.

† Weight used is the average of initial and pre-infection weights.

‡ Weight used is the average of pre- and post-infection weights.

§ Food consumed data is expressed on dry weight basis.

most severe in the animals killed 60 hr post-infection. The cortico-medullary junction and papillary regions of the kidneys were congested in infected animals 5, 8, 11, and 12. The stomach and small intestine were free of ingesta in all dogs and the colon and rectum contained soft, green faecal material.

Due to the acute nature of the study, infection did not result in a significant decrease in body weight. Spleen weights were increased as early as 12 hr post-infection, and the liver weight was variable (Table III).

In the livers of dogs infected 60 hr, there were numerous areas of focal necrosis with mononuclear cell infiltrate similar to those described in rats (McGuire, Young, Payne and Newberne, 1968). The liver lesion was not yet discrete in

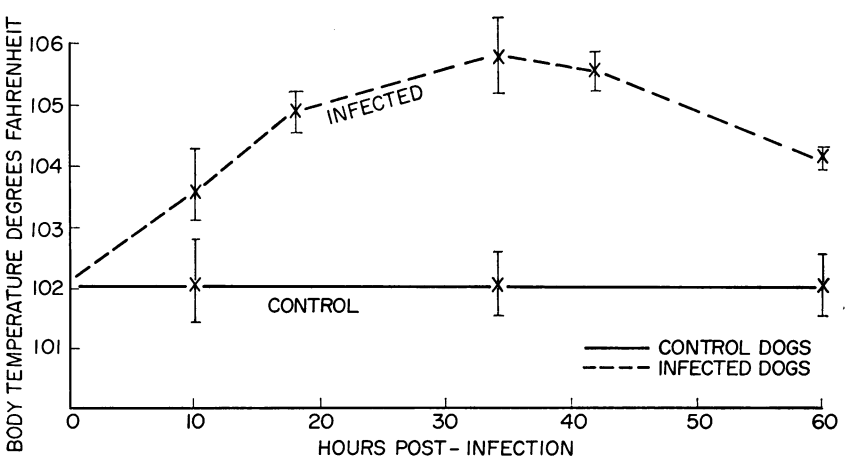


FIG. 1.—Effect of *Salm. typhimurium* infection on body temperature of dogs.

TABLE III.—*Individual Body and Organ Weights of Control and Infected Dogs*

Treatment	Dog no.	Initial B.W.	Pre-infection B.W.	Terminal B.W.	Liver		Spleen		Kidney	
					g.	B.W.	g.	B.W.	g.	B.W.
Control	4	7·6	6·7	6·8	239·2	3·5	13·9	0·20	39·5	0·58
12 hr	5	6·4	6·2	6·2	—	—	15·9	0·25	38·6	0·62
12 hr	6	7·6	7·0	6·9	231·4	3·3	18·1	0·26	40·5	0·58
Control	7	6·8	6·6	6·8	247·4	3·6	12·2	0·18	34·5	0·51
36 hr	8	7·5	6·7	6·4	254·9	4·0	18·5	0·29	33·9	0·53
36 hr	9	6·6	6·5	6·5	232·5	3·6	19·5	0·30	35·7	0·55
Control	10	6·2	5·8	5·7	175·8	3·1	12·8	0·23	27·1	0·48
60 hr	11	6·5	6·1	5·9	226·5	3·8	17·7	0·30	29·3	0·50
60 hr	12	6·2	5·9	5·4	198·2	3·6	15·4	0·28	30·9	0·57

animals infected for only 12 and 36 hr, but increased cellular activity was present. The spleens of dogs infected 60 hr exhibited numerous circumscribed foci of reticuloendothelial cell hyperplasia and perifollicular haemorrhage. Histochemically there were no observed differences in either acid phosphatase or glucose-6-dehydrogenase in the liver of any of the infected groups.

There was a neutrophilia at all times as a result of an increased number of band neutrophils, though by 36 and 60 hr most of the neutrophils were classified as mature (Table IV). Lymphopenia was observed at 12 hr in dogs 5 and 6 which had corrected itself by 60 hr in dogs 11 and 12 with an outpouring of young lymphocytes and an eosinopenia was also observed following infection.

TABLE IV.—*Pre- and Post-infection Blood Values*

Treatment	Hb. g.	PCV	Number of cells per mm³ of blood							
			Leuko-cytes	Mature neutro-phils	Band	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils	Young lympho-cytes
Control	13·1	39·3	9383	5544	269	2404	779	252	28	29
12 hr post-infection	11·9	35·0	20050	6554	12768	722	717	0	0	0
36 hr post-infection	11·7	35·5	10700	7751	1409	984	509	0	0	0
60 hr post-infection	11·0	32·5	15000	8664	2256	2652	900	78	0	435

TABLE V.—*Pre- and Post-infection Serum Protein Values*

Protein fraction	Experimental treatment							
	Control		12 hr post-infection		36 hr post-infection		60 hr post-infection	
	Range of 16 determs. 12 dogs	Mean	Dogs 5, 6	Mean	Dogs 8, 9	Mean	Range of 4 determs. dogs 2, 3, 11, 12	Mean
Total serum proteins	4·80–5·60	5·21	4·90, 5·10	4·99	5·50, 5·90	5·70	5·40–6·10	5·69
Albumin	1·99–2·63	2·40	2·37, 2·39	2·40	2·30, 2·71	2·50	1·78–2·27	2·00
α-1	0·45–0·97	0·71	0·55, 0·63	0·59	0·33, 0·72	0·54	0·20–0·49	0·36
α-2	0·38–0·92	0·62	0·54, 0·55	0·54	0·87, 1·43	1·15	1·28–1·93	1·57
β-1	0·16–0·39	0·28	0·20, 0·33	0·26	0·21, 0·36	0·29	0·24–0·60	0·37
β-2	0·57–0·92	0·72	0·69, 0·75	0·72	0·70, 0·79	0·74	0·66–0·97	0·81
γ	0·35–0·69	0·51	0·48, 0·52	0·50	0·36, 0·61	0·48	0·58–0·69	0·64

Although control dog 10 had increased haemoglobulin and PCV values at the terminal bleeding, control animals 4 and 7 had depressed values but the depression was less than in the infected animals. Dogs 10 (control) and 11 and 12 (60 hr) had lower pre-infection values than the other dogs and the actual decrease in haemoglobin and PCV values observed in dogs 11 and 12 were no greater than those observed in the other infected animals even though the latter two had 24 hr more to respond.

TABLE VI.—*Control and Infected Liver Free Amino Acids* (μM/g.)

Amino acids	Control*	12 hr† post-infection	36 hr† post-infection	60 hr† post-infection
Aspartic acid	0·840	0·920	0·900	0·840
Threonine	0·330	0·390	0·350	0·320
Serine	0·750	0·680	0·660	0·610
Proline	0·360	0·400	0·700‡	0·740
Glutamic acid	1·970	2·460	3·260	3·080
Glycine	0·850	0·840	0·740	0·760
Alanine	2·980	3·080	2·580	2·370
Valine	0·270	0·190	0·330	0·370
Cystine	Trace	Trace	Trace	Trace
Methionine	0·081	0·096	0·094	0·088
Isoleucine	0·140	0·110	0·160	0·160
Leucine	0·260	0·240	0·320	0·280
Tyrosine	0·100	0·095	0·098	0·098
Phenylalanine	0·098	0·094	0·130	0·130
Lysine	0·120‡	0·160	0·220‡	0·240‡
Histidine	0·250‡	0·360	0·420‡	0·390‡
Arginine	0	0	0	0
Totals	9·400	10·120	10·960	10·480

* Mean value of 3 determinations except where noted.
† Mean value of 2 determinations except where noted.
‡ Value of 1 determination.

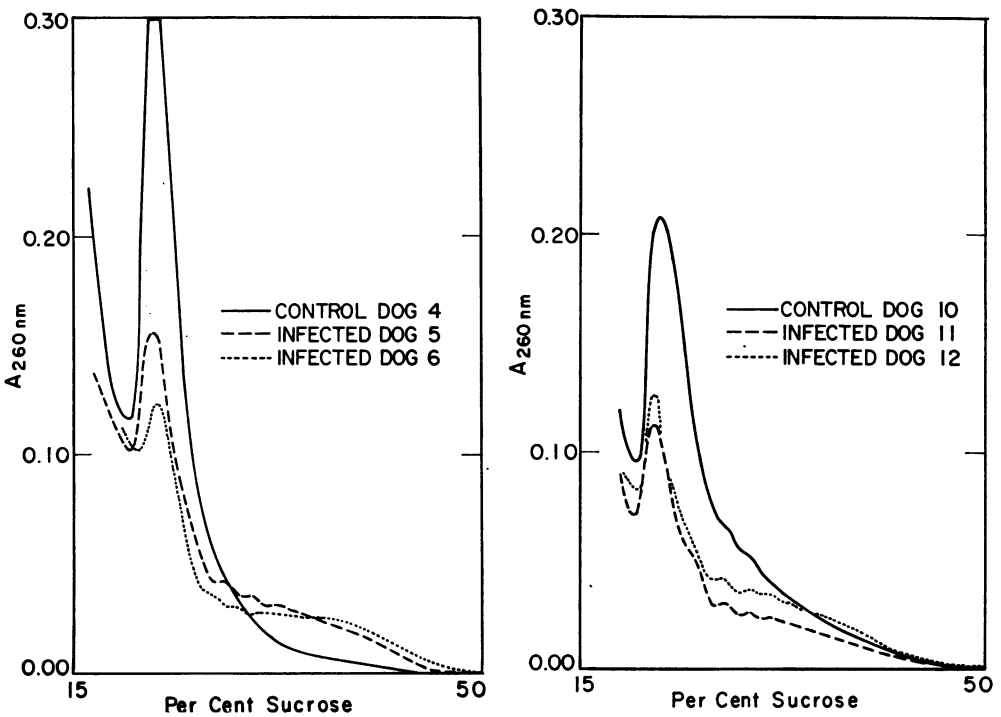
TABLE VII.—*Pre- and Post-infection Serum Free Amino Acids* (μL/ml.)

	Control		12 hr post-infection		36 hr post-infection		60 hr post-infection	
Amino acids	Mean	Range of 12 values	Mean 2 values	per cent of control Mean	Mean 2 values	per cent of control Mean	Mean 2 values	per cent of control Mean
Aspartic acid	0·034	(0·021–0·045)	0·024	70	0·020	59	0·022	65
Threonine	0·171	(0·115–0·220)	0·118	69	0·116	68	0·101	59
Serine	0·212	(0·165–0·342)	0·158	74	0·114	54	0·110	52
Proline	0·122	(0·103–0·135)	0·069	56	0·065	53	0·042	34
Glutamic acid	0·056	(0·039–0·076)	0·054	96	0·034	61	0·032	57
Glycine	0·251	(0·219–0·318)	0·156	62	0·131	52	0·079	31
Alanine	0·306	(0·194–0·459)	0·179	58	0·157	51	0·082	27
Valine	0·108	(0·086–0·118)	0·090	83	0·095	88	0·104	96
Cystine (half)	0·086	(0·052–0·145)	0·038	23	0·050	58	0·063	73
Methionine	0·038	(0·027–0·052)	0·025	66	0·030	79	0·027	71
Isoleucine	0·053	(0·039–0·072)	0·047	89	0·038	72	0·061	115
Leucine	0·090	(0·070–0·125)	0·072	80	0·078	87	0·107	119
Tyrosine	0·029	(0·020–0·039)	0·030	103	0·022	76	0·024	83
Phenylalanine	0·040	(0·033–0·052)	0·058	145	0·052	130	0·063	158
Ornithine	0·022	(0·014–0·031)	0·017	77	0·016	73	0·007	32
Lysine	0·131	(0·090–0·179)	0·080	61	0·075	57	0·056	43
Histidine	0·054	(0·039–0·063)	0·072	133	0·048	89	0·042	78
Tryptophan	0·048	(0·016–0·075)	0·094	196	0·056	117	Trace	0
Arginine	0·154	(0·126–0·198)	0·104	68	0·136	88	0·140	91
Totals	2·005		1·485	74	1·333	66	1·162	58

The α-2 fraction of serum protein exhibited the most marked change; it increased progressively with infection from 36 to 60 hr. The α-1 fraction decreased in both the 36 and 60 hr groups but the albumin fraction was not appreciably affected by infection (Table V).

Mean total liver amino acids were increased in the infected animals compared to mean control values. Individual total liver free amino acid values at each infection period were increased above the control value of the group in all but dog 12 though there was some overlap between control and infected totals among the groups (Table VI) as total liver free amino acids were increased to 10·614 μM/g. in the 46 hr fasted control dog 10.

The total free serum amino acids were depressed in all infected animals, becoming apparent as early as 12 hr post-infection; however, the decrease was most marked in dogs infected 60 hr (Table VII).



FIGS. 2 and 3.—Effect of *Salm. typhimurium* infection on the sedimentation of dog liver ribosomes at 12 and 60 hr post-infection. A 1 per cent detergent (DOC) treated post-mitochondrial supernatant preparation (0·2 ml. of a 33 per cent homogenate diluted 1 : 2) was layered over 12 ml. of a linear (15–50 per cent) buffered sucrose gradient (0·05M tris base pH 7·5 5°, 0·025M KCl, 0·005 MgCl_2). The gradients were centrifuged at 25,000 r.p.m. (40,000 × *g* (min)–100,000 × *g* (max) 0°) for 3½ hr in a B-35 IEC ultracentrifuge with a SB-206 head, a 6 place—14 ml. tube capacity, 96 mm. tube length—swinging bucket rotor. Each curve represents the profile from approximately 22 mg. liver equivalent prepared in duplicate from 5 g. of liver obtained from a single dog. The gradients were monitored at 260 mμ and recorded automatically with an Electronik 15 Strip Chart Chromatography Recorder by puncturing the bottom of the tube and pumping the contents through a 2 mm. flow cell of a Gilford Model 240 Spectrophotometer with a signamotor pump unit which utilizes a Zero-max drive assembly. Twelve and 60 hr polysome profiles are illustrated respectively. The decrease in polysome fractions between 12–60 hr is evident.

Under conditions in which polysome profiles were determined in this laboratory, control animals had a very large monosome peak with very little polysome fraction. Following infection, the monosome fraction decreased and there was a corresponding increase in the polysome fraction, most apparent in animals infected for only 12 hr (Fig. 2). By 60 hr post-infection there was a decrease in the polysome fraction (Fig. 3) of infected animals compared to the 12 hr sample indicating that liver protein synthesis was more active at 12 hr following exposure to infection than it was at 60 hr post-infection.

DISCUSSION

The time of infection (10 p.m.) and interval to death (12, 36, and 60 hr) were chosen so that all animals could be killed on successive days at the same hour. The dogs were fed during the conditioning period a quantity of food which in the past was found to result in a small gain in body weight (Newberne, 1966). This amounted to approximately 450 g./day (150 g. dry matter) to 5 month old male beagles. In this study, however, the animals failed to maintain their body weight when fed this level of intake during the conditioning period. This was equated with a change in the strain of beagles used in the investigation.

The animals killed 60 hr post-infection failed to eat on the second day of infection; thus, they were killed following a 46-hr fast. The 12-hr and 36-hr infection animals all consumed food the day prior to infection and thus were killed 22 hr after the last meal (Table II). The fact that dogs 10, 11, and 12 were killed following a 46-hr fast is of significance. It has been noted that following either fasting or consumption of a protein-free diet 20 per cent to 30 per cent of liver protein, RNA, and phospholipid are lost within the first 2–3 days (Munro, 1968). The loss of liver mass associated with prolonged fasting observed in dog 10 was apparently prevented by infection as dogs 11 and 12 had slightly increased liver mass when expressed as a per cent of either pre-infection or terminal body weight.

The aggregation of polysomes is dependent on diet and on the basis of *in vivo* and *in vitro* studies it appears that deficiency of essential amino acids from the diet or from incubation medium leads to disaggregation of polysomes (Munro, 1968).

Polysome profiles were utilized in this experiment to monitor the early effects of bacterial infection on protein synthesis. Profiles were determined for all 12 dogs, but only representative examples are shown. In all control animals which amounted to 8 profiles, the monosome area accounted for the majority of the 260 mμ absorption; this has been the case in previous work in this laboratory with normal dogs. In profiles obtained from infected animals, a total of 16, the monosome area was decreased and disome and polysome fractions were present (Figs. 2 and 3). Although the profiles obtained from this experiment are not optimum; experimental conditions described were constant and the degree of ribosome aggregation was increased to a maximum as early as 12 hr post-infection. A comparison of dog and rat liver polysome profiles is shown in Fig. 4 to illustrate the difference in the two species under standard conditions in our laboratory.

Subsequent work has shown that increasing the magnesium ion concentration threefold to 0·015M $MgCl_2$ was without effect, but slight modifications in technique introduced to increase resolution and decrease ribonuclease activity resulted in better profiles. In later work the use of the high speed supernatant fraction from rat liver containing ribonuclease inhibitors (Blobel, 1966) improved the profiles

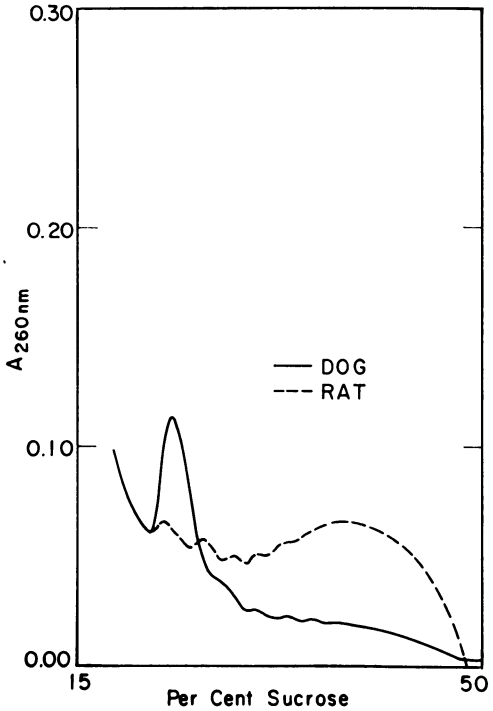


FIG. 4.—Comparison of rat and dog liver polysome profile prepared simultaneously from a 1 per cent detergent (DOC) treated post-mitochondrial supernatant preparation of which 0·4 ml. of a 33 per cent homogenate diluted 1 : 3 33 mg. liver equivalent was layered over 12 ml. of a linear (15–50 per cent) buffered sucrose gradient (0·05M tris base pH 7·5 0°, 0·025M KCl, 0·005M $MgCl_2$). The gradients were centrifuged at 25,000 r.p.m. (40,000 × *g* (min)–100,000 × *g* (max) 0°) for 3½ hr rat, 5 hr dog in a B-35 ultracentrifuge with an SB-206 head, a 6 place—14 ml. tube capacity, 96 mm. tube length—swinging bucket motor. The gradients were monitored at 260 mμ and recorded automatically by puncturing the bottom of the tube and pumping the contents through a 2 mm. flow cell of a Gilford Spectrophotometer. The species difference in polysome profile, as prepared in our laboratory, is interesting and indicates a need for comparative studies with this useful technique.

but they never reached the quality of rat profiles done simultaneously. It is possible that the ribonuclease activity is elevated in dog liver, compared to rat liver, or possibly the RNAase/RNAase-inhibitor relationship is of a different nature from that in rat liver. We have not observed references to this in the accessible literature.

The elevation of the α-2 fraction of serum protein observed 36 and 60 hr post-infection (Table V) has been reported to be associated with a variety of diseases and also following numerous experimental procedures (Chandler, 1968; Macbeth, 1962; Keyser, 1964). Of interest is a study on injury and plasma protein biosynthesis (Chandler, 1968; Liu, 1968). In rats stressed by laparotomy while under ether anaesthesia followed by closure, the incorporation of ^{14}C-orotic acid into RNA was maximal at 8 hr post-injury. There was also an increased aggregation of ribosomes as polysomes which was maximal at 18 hr and at the same time the liver alkaline ribonuclease activity was reduced to 50 per cent of control value. Glycoprotein synthesis as measured by the uptake of glycine-1-^{14}C into isolated serum glycoproteins was also maximal at 24 hr.

The RNAase-inhibitor of the high speed supernatant of rat liver homogenate has been defined as a heat labile nondialysable substance probably glycoprotein in nature (Shortman, 1961; Roth, 1956). The liver has been shown by many investigators using different techniques to be the site of synthesis of proteins within the α-2 fraction which contains a majority of the glycoproteins (Schultze and Heremans, 1966).

A possible explanation for the greater polysome material present in infected dog liver homogenate was the existance of a more favourable RNAase/RNAase-inhibitor relationship as a result of new glycoprotein synthesis by infected dog liver, assuming that the increase in the serum α-2 fraction was a result of new glycoprotein synthesis which had RNAase-inhibitor properties. Liu (see above) in her discussion did not put forth a reason for the 50 per cent reduction in liver RNAase activity, but simply stated that the increased ribosome aggregation may be dependent on a reduced RNAase activity and a greater binding of the ribosomes with the endoplasmic reticulum as membrane bound polysomes are more resistant to ribonuclease than are free polysomes.

The major defence of the mammalian host against circulating gram negative bacteria and associated endotoxin is the reticuloendothelial system. Opsonins (serum factors) have been shown to be involved in activity of the reticuloendothelial system. With respect to various inert particles, it has been demonstrated that selective depletion of the α-2 fraction of serum by barium sulphate absorption markedly interferes with *in vitro* hepatic phagocytosis (Saba, 1966). Not all investigators agree as to the precise identification of natural occurring opsonin(s) (Megirican, 1968). Our work does not clarify any of the above questions, but an attempt is being made to better understand the role of the different proteins in the α-2 fraction and to elucidate their possible role in host resistance. We have observed, however, that selected nutritional deficiencies appear to sharply limit the capacity of the reticuloendothelial system in rats (Newberne, Hunt and Young, 1968).

In this study individual chromatograms were done on each serum sample pre- and post-infection and on each liver sample post-infection. The ninhydrin positive substances quantitated include 18 amino acids common to most proteins plus ornithine. Glutamine and asparagine which elute together were not quantitated. Work is still in progress to improve sample preparation, resolution and identification of ninhydrin reacting substances in serum and tissues. By monitoring ninhydrin reactive components of serum and liver it was felt that a characteristic pattern might develop which would allow us to follow metabolic alterations in the early stages of infection and to possibly show differences between viral and bacterial infection. This subject is under continuing active investigation in our laboratories.

In general as a result of infection, there was a depression of total serum free amino acids which was apparent at 12 hr with some exceptions; tryptophan, histidine, and phenylalanine were increased and the branched chain amino acids were unchanged. Post-infection total serum free amino acid values of all infected animals were less than their pre-infected values (Table VIII). In each case the dog which appeared to be the most severely depressed clinically and which had the greater elevation of temperature also had the greatest depression of total serum free amino acids. The depression was due in large part to the non-essential amino acids serine, glycine, alanine, and proline; of the essential amino acids lysine

TABLE VIII.—*Per Cent Depression of Serum Amino Acids*

Treatment	Dog no.	Pre-infection/ Post-infection (per cent)	Amino acids below 80 per cent
12 hr Control	4	92	0
12 hr Infected	5	55	15/18
12 hr Infected	6	87	4/18
36 hr Control	7	109	0
36 hr Infected	8	55	14/18
36 hr Infected	9	77	7/18
60 hr Control	10	99	0
60 hr Infected	11	55	15/18
60 hr Infected	12	68	9/18

and threonine contributed most to the depression. Serum tryptophan and histidine values were decreased by 36 and 60 hr, but phenylalanine remained elevated throughout the entire periods of observation.

In dog 5 the individual branched-chain amino acids were each depressed below 80 per cent of their pre-infection control values, but were unchanged from control values in infected dogs 6, 8 and 9 and in control dogs 4 and 7. At 60 hr post-infection valine, isoleucine and leucine were increased in dog 12 to 123, 161 and 149 per cent respectively of the pre-infected control values for this dog. The values for these amino acids in infected dog 11 which was more severely infected clinically were 90, 68 and 90 per cent respectively of their pre-infection value. The 46 hr fasted control had elevated values for valine of 130 per cent, isoleucine of 123 per cent and leucine of 114 per cent.

Except for alanine which decreases markedly at 36 and 60 hr and serine and glycine which were decreased slightly, there was an increase in total liver free amino acids. Glutamic acid accounted for most of the increase although phenylalanine was increased at both 36 and 60 hr.

Hydrocortisone is known to enhance the intrahepatic accumulation of amino acids from extra-hepatic tissue (Feigelson and Feigelson, 1964). The effects of prolonged fasting and infection both stress the animal; the separate effects of these will be difficult to sort out since both evoke an alteration in hormone balance which is variable among dogs.

In our studies the animals of each infection period (12, 36, and 60 hr) with the highest temperature also had the greatest depression of serum amino acids. In the case of 36 and 60-hr periods, infected dogs also had the greatest increase in liver free amino acids. As in distemper-infected dogs, the phenylalanine/tyrosine ratio (P/T) was increased and, as above, the dogs with the greatest febrile response also had the greatest elevation of the P/T ratio. In the distemper study (Newberne, 1966) obese animals had a greater elevation of the P/T ratio than the slightly underfed dogs.

It would appear from studies thus far that the dogs with the most severe clinical signs also have a greater elevation of the P/T ratio; this finding is being checked more closely as to its significance.

The nature of the amino acid changes discussed in this paper are not of diagnostic significance, but further study of ninhydrin-positive substances from serum, liver, muscle, and urine may help to better understand the response to infection. Since branched chain amino acids (valine, leucine, and isoleucine) are metabolized in a manner different from other amino acids and since alanine and glutamic acid

are directly involved in the tricarboxylic acid cycle further studies may shed light on the interrelationships between infection, and aerobic and anerobic metabolism in the cell and lead to convenient measurements which will aid in prevention or control of infections early in the course of a disease. Since this study was initiated, other investigators, using gas chromatography, have presented evidence that a new metabolite appears in the serum of dogs injected with canine hepatitis virus (Mitruka, 1968). In dogs that survived, the new component began to disappear at about the seventh day but it remained in those dogs which died, along with a second new peak which was not seen in dogs surviving. The study was extended to tissue culture, and the virus of canine hepatitis plus two additional viruses were investigated in dog kidney cells. It was observed that of the three viruses studied each gave rise to components which were unique to the infecting virus as well as similar to all three, and that the tissue culture work with canine hepatitis agreed with the intact animal findings.

Results of our studies as well as those of others support the hypothesis that clinical parameters will become available for measurement which will indicate biochemical changes early on in disease processes which are amenable to reversal through therapy.

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