for use in repeated experiments. Dog 1 (9.2 kg) was given 2 injections of 0.6 ml/kg each, without the production of fibrillation. With artificial respiration continued, 2 mg of methacholine chloride were given intravenously 17 minutes after the second injection, resulting in the appearance of auricular fibrillation with prolonged A-V block and later of markedly abnormal ventricular complexes. Death followed in about 5 minutes. A dose of 2 mg of methacholine chloride is rarely fatal in otherwise untreated dogs under pentobarbital anesthesia. Dog 2 (11.2 kg) received 5 injections totalling 3.0 ml/kg, the largest being 1.0 ml/kg without production of fibrillation. Two subsequent doses of 1.2 ml/kg each produced fibrillation. Death resulted after the respirator was disconnected one minute after the last injection. In dog 3 (12.7 kg) given 0.70, 0.75 and 0.70 ml/kg successively, fibrillation resulted after the second dose only. The animal died 15½ hours later and at necropsy showed gastric dilatation, pulmonary congestion and marked intestinal hemorrhage. In dog 4 (5.8 kg) administration successively of 0.17, 0.35, 0.69 and 0.52 ml/kg resulted in the production of auricular fibrillation only with the last 2 doses. The animal remained anesthetized for about 36 hours and thereafter had marked ataxia and cough for a week. In a second experiment 16 days later the minimal fibrillating dose was 1.1 ml/kg, determined after a total of 3.5 ml/kg had previously been given and after a dose of 1.0 ml/kg had failed to produce fibrillation. The animal died 2 days later without recovering from Dowanol anesthesia, 4.6 ml/kg total having been given during the last experiment.

Summary and conclusions. When administered intravenously to dogs anesthetized with pentobarbital, Dowanol 50B in adequate dosage produces auricular fibrillation, but in 4 dogs was found unsuitable for use in repeated experiments on different days, since it caused respiratory depression, prolonged anesthesia, disturbances of gait, intestinal hemorrhages and death. One animal surviving a first experiment required a significantly higher single dose and higher cumulative dose for production of fibrillation during a second experiment and succumbed following the latter.

We wish to thank the Dow Chemical Co. for a generous supply of Dowanol 50B.

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Received June 25, 1952. P.S.E.B.M., 1952, v80.

Effect of Liver Ischemia on Plasma in the Dog, as Measured by Electrophoretic Analysis.* (19700)

A. M. RAPPAPORT, D. W. CLARKE, AND M. STEWART. (Introduced by C. H. Best)

From the Department of Physiology, University of Toronto.

Early attempts to study changes in the plasma proteins of dogs after hepatectomy were hampered by the short survival time of the prepared animals. Munroe and Avery(1) found no significant changes in the electrophoretic pattern of dog plasma, in periods up to 7 hours after hepatectomy. Lewis, Page, and Reinhard(2) were able to obtain plasma samples up to 16 hours after the operation. They reported but small changes. The total plasma protein fell, and a decrease in the β-globulin accounted for much of this change. We know of no reports of electrophoretic studies on plasmas of dogs with reduced hepatic circulation. Although Eck fistula dogs have been studied from a hematological viewpoint, there is little known about the electrophoretic patterns of their plasmas.

Our studies have been made on dogs which, strictly, were not Eck fistula dogs, although a portal-caval anastomosis and ligation of the portal stem close to the liver had been carried out in all of them. To obtain a maximum reduction of circulation in their livers, the dogs were subjected to a 3-stage operation in which, ultimately, all of the named vessels

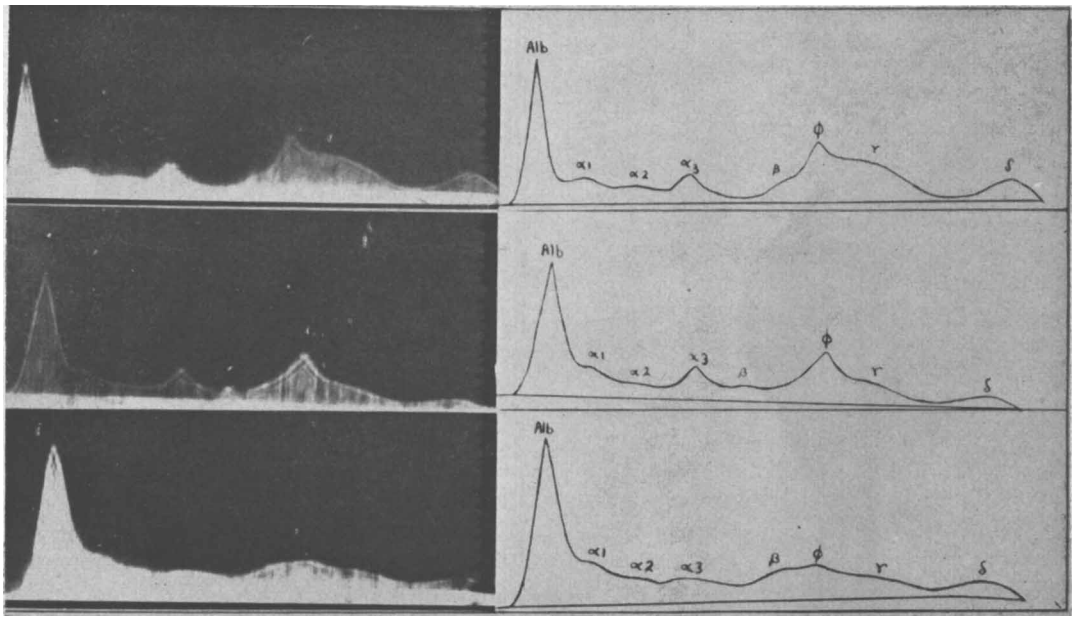

FIG. 1. Tracings of electrophoretic patterns of dog plasma, 2-3 months after stage 2. Top, dog A22; center, dog A9; bottom, normal dog.

which supplied the liver were completely tied off. The organ continued to live on collateral blood supply alone(3).

After the first and second stages of surgical preparation, the hepatic artery and all its branches are completely tied off, and the portal blood is in part diverted from the liver by a partial Eck fistula. This fistula consists of a portal-caval anastomosis and a ligation of the portal vein below its pancreatico-duodenal tributary. Venous blood from the pancreas, duodenum and stomach can still flow into the liver through the pancreatico-duodenal vein.

In the third stage of the operation, the pancreatico-duodenal vein was ligated, with the result that portal blood was totally diverted from the liver. The hepatic circulation in these dogs is now maintained mostly by arterial collaterals from the left gastric and phrenico-abdominal arteries. They supply the liver with arterial twigs which penetrate through the coronary ligaments and subserously through the hepato-duodenal ligament.

For the electrophoretic studies, 3 ml of heparinized plasma from the experimental animals were diluted up to 12 ml with barbiturate buffer, of pH 8.6 and ionic strength 0.1. The diluted plasma was dialysed against one liter of buffer for 18-20 hours at 0°C. Analyses were made in the standard analytical cell of the Aminco-Stern electrophoretic apparatus. With a potential gradient of 5.1-7.4 v/cm, satisfactory resolution of the plasma proteins took place in 60-90 minutes. Ascending patterns were analyzed to determine the relative concentrations of the various proteins. Total protein was determined by the micro-Kjeldahl method.

Observations and results. Significant changes in the electrophoretic pattern appeared only after the first and second stages of the surgical preparation had been completed. The plasma of such dogs showed a slight decrease in the albumin fraction and an increase in the sum of the $\beta + \phi + \gamma$ fractions. (Fig. 1 and Table I). The values of albumin recorded as normal in Table I may appear low, but this is found to be the average value for dogs on a "fox chow" diet. The A/G ratio is lower than normal.

In 3 other dogs (Fig. 2 and Table I) the plasma was analyzed 2 to 3 weeks after the third stage of the operation. The albumin is lower in these dogs than after the second

TABLE I.

	Albumin	α_1	α_2	α_3	β	ϕ	γ	A/G	Total protein, g %
Normal	39.2	11	7.3	11.9	⌊——30.7——⌋			.64	7.66
A9	36.7	⌊—13.9—⌋		11.1	3.2	⌊—34.9—⌋		.58	5.7
A22	27.6	9.7	6.8	9.7	⌊——46.2——⌋			.38	6.13
A30	24.4	⌊—14.5—⌋		9.8	⌊——51.4——⌋			.32	5.55
A49	9	5.8	7.1	8.3	⌊——69.8——⌋			.099	6.6
A50	18.6	15.6	4.5	12.6	⌊——48.7——⌋			.23	5.94

stage, and much lower than normal. The corresponding increase in globulin seems to have occurred in the $\beta + \phi + \gamma$ fractions. The A/G ratio is seen to be very low—dropping to 0.099 in the case of dog A49.

Analyses of plasma samples obtained several months after the different operative stages showed the following results, which are also summarized in Table II.

Dog A50 (see also Table I), 6 months after stage 3, has not recovered a normal albumin value (Fig. 3), but instead, the albumin has dropped slightly.

Dogs A35, A46, A48, 5 to 6 months after stage 3 (Table II), have more normal values of albumin. Apparently the collateral circulation has increased to assure normal protein production by the liver. It might be noted that Dog A48, though in a precarious state of nutrition and menaced by slight bouts of meat intoxication, still has an almost normal albumin level. It should be mentioned that all dogs have been maintained on a constant diet at all times.

That the condition of the dogs is at least as important as the time factor is seen from a

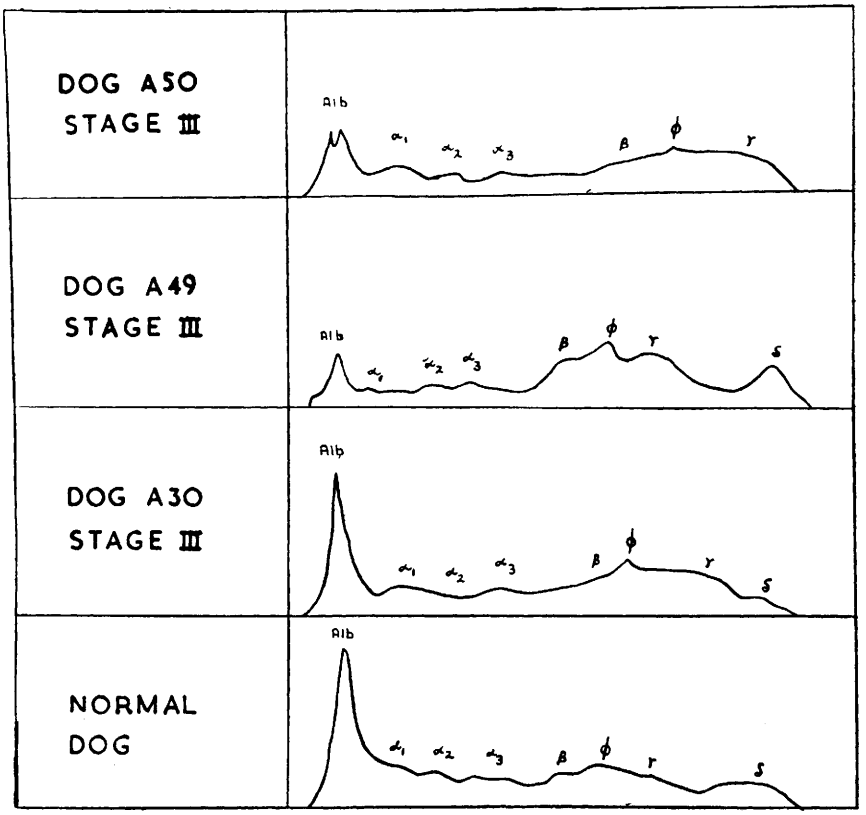

FIG. 2. Tracings of electrophoretic patterns of dog plasma, 2-3 weeks after stage 3.

TABLE II.

Run	Dog	Albumin	α_1	α_2	α_3	β	ϕ	γ	A/G
168	A50	15.7	3.5	6.3	13.4		37.7	23.4	.28
169	A35	41.1		10.4	12.7	6.2	18.3	11.2	.70
170	A46	35.1	12.6	6.4	6.7	11.3	19.9	12.1	.54
178	A58	40.7		20.4	12.2		26.2		.69
179	A48	34.6		11	21.6		32.6		.53
182	A55	28.6		10.6	22.3		37.8		.40
219	HC34	41.2	3.9	9.7	11.7	8.4	13.3	11.7	.70
207	HC28	37.7		14.2	16.2	7.9	13.5	10.7	.60
209	HC27	31.7		15.2	20	9.9	12.9	10.3	.46

consideration of the results after stage 2.

Dog A58, 6 months after stage 2, has a normal albumin level, whereas Dog A55, 7 months after stage 2, has a low albumin value (Fig. 3 and Table II). However, this latter dog had a very difficult recovery after stage 1, complicated by perforation of the gall bladder and generalized peritonitis. Although the recovery after the second stage was uncomplicated, still the animal had a prolonged convalescence with poor food intake.

Dog A9, 2 months after stage 2, had a normal albumin level whereas Dog A22, 3 months after the same operation, had a low albumin value (Table I). This dog went through several severe bouts of "meat intoxi-

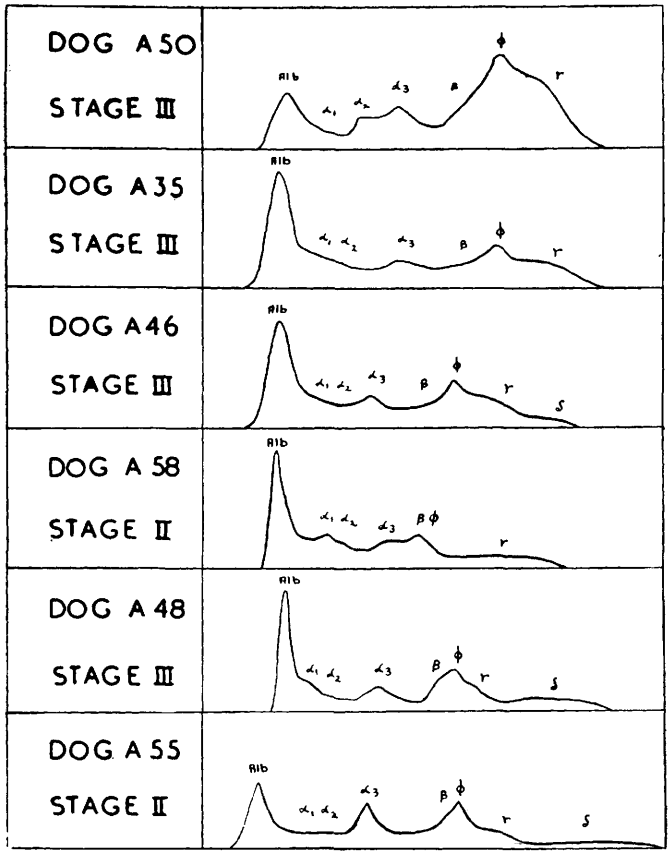

FIG. 3. Tracings of electrophoretic patterns of dog plasma, 6-7 months after stages 2 and 3.

cation" from which it recovered with difficulty. There is the impression that massive doses of vit. A were of some help. (This therapy was suggested by the observation of signs of vit. A deficiency, such as a scaly skin, sublingual ulcers, blindness, reduced sensitivity of the skin, etc.).

Two dogs (HC-27 and HC-28, Table II) in a state of hepatic coma produced by ligation of the common hepatic artery, combined with a classical Eck fistula(4) showed albumin percentages of 31.7 and 37.7, *i.e.*, not markedly low values. One animal, HC 34 (Table II), which had recovered from hepatic coma and was still alive after 13 months, showed a normal pattern.

Summary. Successive surgical interference with the blood supply to the liver is followed by a decrease in the total proteins. The greater the interference with the circulation, the lower is the albumin level. The higher globulin level is due to an increase in the $\beta + \phi + \gamma$ fractions. It is suggested that a longer lapse of time after the experimental reduction of the blood supply permits the establishment of an adequate collateral circulation and tends to reestablish the normal production of proteins by the liver. In acutely induced fatal hepatic coma no large changes in the electrophoretic patterns of the dog plasmas were observed during the short survival time.

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* This work was partially supported by a grant from the National Research Council of Canada.

Received June 26, 1952. P.S.E.B.M., 1952, v80.

Isolation of *M. tuberculosis* by Inoculation of the Yolk Sac of Embryonated Eggs.* (19701)

JOHN W. BRUECK AND G. JOHN BUDDINGH.

From the Department of Microbiology, Louisiana State University School of Medicine, New Orleans.

Previous investigators(1,2) have demonstrated that *M. tuberculosis* proliferates with great rapidity in the yolk sac of embryonated eggs. These observations were made on embryos inoculated into the yolk sac with pure cultures of the microorganism. Careful search of the literature gives no indication that the yolk sac has been utilized for the rapid isolation of *M. tuberculosis* from materials collected from patients for the purpose of a more rapid laboratory diagnostic procedure. Investigations which are being performed in this laboratory indicate that inoculation of the yolk sac of chick embryos provides a rapid means for establishing the etiological diagnosis with materials from patients with suspected tuberculosis. A preliminary report of the results obtained thus far appears to be warranted.

Methods. Age of embryos and method of inoculation. Embryos of 5 to 8 days preliminary incubation are suitable. The yolk sac is inoculated through a small drill hole in the shell by means of a syringe and 20- or 22-gauge needle. After injection the drill hole is covered with melted paraffin.

Preparation preliminary to inoculation of materials tested. Only such materials collected from patients were tested in which a thorough search by the established methods, including concentration procedures, failed to reveal the presence of acid fast bacilli by microscopic examination. Six embryos each were inoculated into the yolk sac with each specimen.

Spinal fluid. Spinal fluid collected under aseptic precautions requires no preliminary treatment. The amount of fluid available determines the size of the inoculum which can vary from 0.2 to 0.5 ml per yolk sac.

Pericardial and pleural exudates. A preliminary test by inoculating 0.2 ml amounts

* Aided by grants from the Eli Lilly Co.