Normal Values of Swine Serum Proteins.* (23696)

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Previous reports of normal swine serum and plasma proteins have indicated a slight disparity concerning total protein concentrations and the numbers and values of constituent fractions. Heretofore these analyses utilized the moving boundary method of electrophoresis. The results of this study provide statistically reliable swine serum protein values as determined by the biuret and zone electrophoretic methods.

Methods. The blood serum samples were obtained from 79 hogs at the time of slaughter. The animals' ages varied between 5½ and 6½ months, comprising a random class regarding size and sex. The hogs were "buyers' Grade 1 and 2." They represented a geographical rearing area including the states of Nebr., Colo., and N. Mex. Total serum proteins were determined by the biuret method(1). A sample contained 0.2 ml of serum, 4.8 ml of 0.85% saline and 5 ml of biuret reagent. The samples were incubated in a 50°C. constant temperature water bath for 30 minutes. An optical density comparison with a bovine plasma albumin standard (Armour) was recorded on the Beckman B spectrophotometer at a wave length of 550 mμ. Procedure for determining serum protein fractions involved the use of a horizontal strip paper electrophoresis apparatus(2). The supporting strip consisted of Whatman #1 filter paper, one inch in width. Serum was applied by two methods. In the one procedure, 0.02 ml of serum was pipetted onto the edge of a glass microscope slide and placed transversely to a predetermined section of the filter paper strip. The other method utilized filter paper, 1 mm wide and 2.2 cm long, saturated in serum and positioned on the supporting strip for the duration of electrophoresis(3). The latter procedure was preferred because it produced more consistent results between numerous analyses and allowed greater ease in applying a uniform distribution of serum. Electrophoretic fractionations were effected in barbital buffer, (pH 8.6 u = 0.1) after 16 hours at 120 volts and 2 ma. Upon completion of the separation, the paper strips were stained for 10 minutes in a saturated Ponceau 2R solution containing 1 part acetic acid, 4.5 parts methyl alcohol and 4.5 parts distilled water. The unbound dye was removed by successive washings in the above mixture. The strips were air dried and the stained protein fractions were cut out and eluted in 5 ml of 0.1N NaOH. The optical densities were recorded at a wave length of 465 mμ. For evaluation purposes, many strips were quantitated with the Spinco Analytrol automatic recording densitometer, prior to the spectrophotometric determinations. Albumin-globulin (A/G) ratio was calculated from the electrophoretic data. Albumin was also salted out with 23% Na_2SO_4, (Howe fractionation) and an A/G ratio obtained by this method(4) was compared with the electrophoretic measurements. The statistical computations were performed by the use of standard formulae(5).

Results. The protein patterns were well resolved into the albumin, alpha, beta and gamma globulin fractions. The constituent protein concentrations were derived from relative percentage values and are included in Table I. The numerous paper electrophoretic separations of swine serum conducted in this laboratory have failed to indicate the presence of an $alpha_1$ globulin which has been previously reported(6,7,9,11). The albumin-globulin (A/G) ratio as derived from the electrophoretic measurements gave a mean value of 0.88 ± 0.025 (one standard deviation). The standard error was 0.003 with 95 and 99% confidence limits of 0.005 and 0.007 respectively. Results from 20 samples of albumin determinations by Howe fractionation disclosed less than a 0.2 g% difference when

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TABLE I. Percent* and g/100 ml of Total Serum Proteins.

	Albumin	α	β	γ	
		(%)			g/100 ml
Mean	46.0	20.0	14.5	19.5	7.4
Stand. dev.	± 5.8	4.0	1.6	4.3	.6
Stand. error	± .7	.4	.2	.5	.1
t_{95}	± 1.3	.9	.4	1.0	.1
t_{99}	+ 1.7	1.2	.5	1.3	.2
			g/100 ml		
Mean	3.4	1.5	1.1	1.4	7.4

* Percentage values were derived from optical density measurements of each fraction, *i.e.* $\% = \frac{\text{O.D.}}{\Sigma\ \text{O.D.}}$

t_{95} = 5% probability level; t_{99} = 1% probability level.

compared to the albumin values derived by the electrophoretic method.

Discussion. These analyses provided statistically reliable total serum protein determinations, including constituent composition values. Although there are reports of per cent protein content of swine serum(6,7,8) only Cartwright *et al.*(9) specifically indicated a mean serum total protein value of 6.70 g/100 ml, as determined from 5 animals. The Handbook of Biological Data(10) reports a mean total plasma protein value of 8.7 g/100 ml, ranging from 7.9 to 10.3.

It would be hazardous to derive valid serum protein values of individual components from plasma studies. Koenig and Hogness (6) found that fibrinogen, when isolated and subjected to electrophoresis, was contaminated with various globulin components and a small amount of albumin, Deutsch and Goodloe(11) stated that fibrinogen comprised 13.9% of the total plasma protein. If this factor is subtracted from the gram per cent of total plasma proteins(10), the resulting value is in excellent correlation with the total serum protein reported in this paper. This study also supports other reports(6,7) regarding per cent composition of serum proteins.

The fast moving component "f" of pig serum detected by Deutsch and Goodloe(11) and named rho by Williams and Grabar(12) is not discernible by this method of zone electrophoresis. However, the "f" fraction has been isolated consistently in this laboratory using a 1% agar as the supporting medium for separation of pig serum proteins.

The existence of an alpha$_1$ globulin fraction has been noted in several reports(6,7,9,10,11), comprising from 3 to 6% of the total proteins when the isolation was performed in a veronal buffer, pH 8.6. The method of zone electrophoresis employed in this study, using a comparable buffer and pH, did not evince the alpha$_1$ fraction on the paper strip. Moore(7) utilizing a moving boundary apparatus, detected an alpha$_1$ with a barbiturate buffer pH 8.6, but failed to do so in a phosphate buffer of pH 7.4. Svensson's(8) moving boundary patterns also failed to disclose an alpha$_1$ globulin. The buffer used was phosphate, pH 7.7, which may account for the absence of this fraction as shown by Moore(7).

Evaluation of the data from this study, reflected upon the serum and plasma values of previous investigations(6,7,8,11) substantiates the probability of the alpha$_1$ fraction migrating with albumin. It is evident that salted out albumin contains the alpha$_1$ globulin, as shown by the resulting concentration being in similitude with albumin derived electrophoretically when alpha$_1$ has not been resolved as a separate fraction or by adding the alpha$_1$ value, when separated, to the albumin factor.

Summary. 1) Zone electrophoretic examination of sera from a large, random selection or apparently healthy hogs has yielded statistically valid protein concentrations. 2) Under the conditions of this experiment, only albumin, alpha, beta, and gamma globulin were resolved and quantitated. 3) The "f" fraction (11) and alpha$_1$ globulin are discernible only under specific conditions. The ionic strength, the pH of the buffer and apparently the separating medium influence the resolution of the "f" and alpha$_1$ fractions.

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Effect of Sex Hormones on Ethanol Induced Fatty Infiltration of Liver in Rats. (23697)

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It has been previously reported that either chronic or acute ethanol intoxication causes lipid to accumulate in the liver of rats(1,2), and that under the same conditions of acute ethanol intoxication, female rats show a more severe fatty infiltration of the liver than do males(2). The experiments reported below were therefore carried out in order to determine the effect of male and female sex hormones on alcohol-induced fatty infiltration of the liver.

Procedure. Eight groups of male and 6 groups of female albino rats, weighing 70-80 g at the start, were maintained on Purina dog chow pellets *ad libitum* for periods of either 4 or 6 weeks. Three of the groups of males and 2 of the groups of females were castrated at the beginning of the experiment. During these periods of time, two groups of intact males received daily intramuscular injections (0.1 ml) of estradiol benzoate in sesame oil which was diluted with Wesson oil, and one such group received daily injections of 0.1 ml of Wesson oil alone. The dose of estradiol benzoate was 0.005 mg/day during the first 2 weeks, and 0.01 mg/day during the last 4 weeks. Testosterone propionate in oil (0.1 ml) was similarly administered to 1 group of castrated males, the dose being 0.1 mg/day during the first 2 weeks and 0.2 mg/day during the last 2 weeks, and to 2 groups of intact females, the dose being 0.1 mg/day during the first week, and increased weekly to 0.4 mg/day. The remaining groups received no injections. All animals weighed approximately 200 g at the end of the 4- or 6-week periods, when they were killed by decapitation. The longer period was required only for those rats receiving estrogen, because the hormone, in the dosage given, was found to exert a small inhibitory effect on the rate of gain of weight. At the end of the 4 or 6 weeks, the rats in 8 of the groups received a single intoxicating dose of ethanol (4.0 ml of a 50% solution by volume) by stomach tube, while those in the remaining groups received a single dose of glucose, calorically equivalent to the dose of alcohol, in the same volume of solution, and in the same manner. All food was then withdrawn, the female rats killed 18 hrs and the male rats 16 hrs thereafter. The livers were immediately removed, samples of liver frozen and subsequently analyzed for total lipids as already described(2). The 18- and 16-hour intervals subsequent to stomach tubing were chosen because it was previously found that female and male rats show peak depositions of liver lipid, subsequent to ethanol intoxication, at these times(2). The seminal vesicles were also removed from 6, and testes from 3 of the groups of males at the time of killing. The fluid was expressed from the seminal vesicles, and these organs as well as the testes weighed on a Roller Smith balance.