TABLE I.
-SH Content of Homogenates of Rabbit, Rat, and Mouse Livers (in γ/100 mg wet weight).

Liver of	Anson's method	Author's method
Rabbit	46.5	45.2
Rat	46.5	47.5
"	48.4	53.8
"	35.6	36.7
"	38.9	36.7
"	47.5	48.6
"	44.3	43.2
Mouse	34.5	35.6
"	31.3	36.5
"	52.9	59.4

Tissues were homogenized in 0.9% NaCl and diluted to a final tissue concentration of 0.5% for Anson's method and to 5% for the present method.

TABLE II.
-SH Content of Kidney, Heart, and Brain Homogenates (in γ/100 mg wet weight).

Tissue	Anson's method	Author's method
Rat kidney	25.2 (18.2-26.6)	56.4 (53.8-60.2)
" heart	20.1 (17.2-26.6)	48.9 (45.2-56.9)
" brain	15.6 (11.8-18.2)	59.2 (55.9-62.3)
Mouse kidney	20.4 (14.0-22.6)	54.3 (35.0-61.3)
" heart	17.2 (10.8-19.4)	41.9 (36.6-47.3)
" brain	14.0 (9.7-16.0)	52.7 (44.1-53.8)

Each figure represents the avg of determinations obtained in 6 animals. Min. and max. values in parentheses. Every determination carried out in duplicate on 2.5, 5, and 7.5 mg tissue samples with Anson's method and on 5 and 10 mg samples with the present method.

0.88 M sucrose, distilled H_2O, 10% urea (in H_2O), and 10% Duponol PC (Na-laurylsulphate), gave 29, 31, 47, 77, and 89 γ of -SH, respectively, per 100 mg of wet tissue. In all tissues analyzed, the lowest -SH values were obtained when NaCl or sucrose solutions were used as homogenizing media, an increase being noted with distilled H_2O or protein denaturing agents. The liberation of -SH radicals in protein denaturation is described by Anson(5).

Comparison showed acceptable agreement with results obtained by the ferricyanide procedure of Anson(6) on liver tissue (Table I), but higher values on kidney, brain, and heart (Table II). The discrepancy may be explained by the protein denaturing effect of amyl acetate on tissue proteins other than liver. The resistance of liver proteins to amyl acetate remains unexplained at present. Native egg white which showed no free -SH radicals with Anson's method(6) reacted with the red -SH reagent, indicating that protein denaturation had occurred during treatment with the amyl acetate solution.

The convenient procedure described in this paper offers the possibility of determining -SH groups in tissue homogenates. The optically clear solutions used for colorimetric measurements give it an advantage over procedures where the color of a turbid suspension has to be estimated. In further experiments, correlation between enzyme activities and -SH content of tissue homogenates is being investigated.

Summary. A colorimetric procedure was developed for the determination of -SH groups. The method involved the estimation of the amount of an organic mercury salt removed from its amyl acetate solution by shaking with tissue homogenates. The dye removed was found proportional to the -SH content of the sample analyzed.

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Hemolysis of Erythrocytes Incubated in Salines. (17869)

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The increase in fragility of erythrocytes caused by incubation of blood at 37°C for 12 to 32 hours, after which treatment the corpuscles may hemolyse even in serum or isotonic saline, has been shown to have differential value between bloods from normal

TABLE I.
Incubation Hemolysis of Dog Blood at 37°C.

	Initial osmotic hemolysis, 1 hr		Hemolysis due to incubation					
			5 hr		9 hr		22 hr	
% NaCl	.41	.70	.41	.70	.41	.70	.41	.70
M %	22*	n	3	6	14	23	47	77
t		—	1.67		2.53		4.12	
P		—	.10		.02		.001	
		—	n.s.		s.		v.s.	

M = Mean of 22 cases.
n = Negligible.
* Deducted from later readings of hemolysis in 0.41 NaCl.
n.s. = not significant.
s. = significant.
v.s. = very significant.

and hemolytic jaundice(1). This increase in fragility can be prevented largely by resuspending the corpuscles in fresh serum every 2 hours during the incubation period (1). In the present studies of hemolysis during incubation, 1:100 blood dilutions made up with salines of different osmotic tonicities were used to minimize effects of the serum. Within the gamut investigated it was found that the greater the tonicity of the saline the greater the incubation hemolysis.

Material and methods. Blood taken from dogs held under favorable conditions and fed standard rations was used. The blood was drawn under aseptic conditions. Quantities to make 1:100 dilutions were discharged at once into pyrex flasks containing saline solutions buffered to pH 7.35 with disodium phosphate(2) and held at 25°C. No anticoagulant was used. The sodium chloride was tested by the method of Ball(3) and found free from silver and other hemolytic substances. Five ml units of the diluted blood were pipetted at once into 30 ml pyrex test tubes which were stoppered with cotton plugs and placed in electrically controlled (±0.1° C) water baths. The tubes were not disturbed until they were removed for analysis. The experimental hemolysis and the total hemolysis were determined for each tube from readings made with a spectrophotometer set at 5450 Å U.

Results. The first group included 22 samples of dog blood, using both 0.41 and 0.70% sodium chloride solutions as the diluting fluids. All of the samples of blood tested showed some hemolysis (average 22%) during the first hour after dilution with 0.41% saline. As there was no significant difference in the amount of this initial hemolysis in series held at 25° and at 37°C, the hemolysis during the first hour was due apparently to the tonicity of the saline and was deducted from subsequent readings, to obtain the hemolysis due to incubation. The hemolysis during the first hour at 25° or 37°C was negligible in dilutions made with 0.70% or stronger salines.

In Table I the means of the hemolyses due to incubation at 37°C are presented, together with the significance of the differences between these means as determined by Fisher's "t" procedure(4). It can be seen that the incubation hemolysis in the 0.70% saline exceeded that in the 0.41% at the end of 5 hours and maintained that relation throughout the remainder of the incubation period. However, the slight difference at the end of 5 hours is not statistically significant and the individual data showed that there was much variation in the time of the onset of incubation hemolysis. By the ninth hour of incu-

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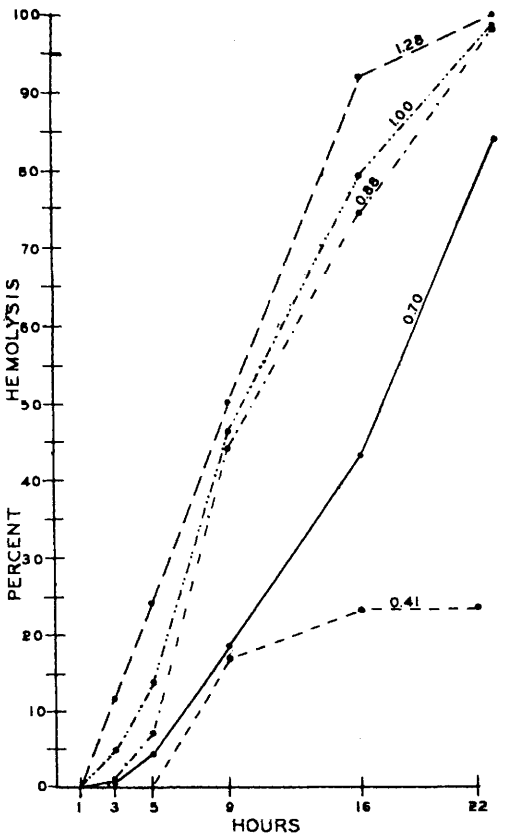

FIG. 1.

Mean % of hemolysis due to incubation at 37°C of 14 samples of dog blood in 0.41, 0.70, 0.88, 1.00, and 1.28% salines. The mean initial osmotic hemolysis at the end of the first hour, 37% has been deducted from the 0.41% series. There was no initial osmotic hemolysis in the other salines.

bation, however, the difference between the 0.41% and 0.70% series was definitely significant with P=0.02 and almost within the P=0.01 grouping. The difference between the 2 series is highly significant after 22 hours incubation, with P=0.001.

In 14 subsequent series of dog bloods the dilutions were made with 0.41, 0.70, 0.88, 1.00, 1.28% salines. The mean values are shown in Fig. 1. The hemolysis at the end of the first hour of incubation at 37°C in the 0.70 to 1.28% salines inclusive was negligible. From the second hour to the end of each incubation test the rate of hemolysis and after a time, the percents of incubation hemolysis and finally the observed hemolysis (incubation hemolysis plus initial first hour hemolysis) in the 0.41% saline were exceeded by the hemolysis in all of the salines of greater tonicity. In addition, the hemolysis in each of the several salines having negligible hemolysis during the first hour, in turn exceeded the hemolysis in all of the salines of lower tonicity.

Discussion. Ham and Castle(1) found that mammalian erythrocytes during incubation at 37°C exhibited pre-hemolytic sphering and it has been stated repeatedly(1,5,6,7) that the more nearly spherical the erythrocytes the greater their susceptibility to hemolysis. As the lower the osmotic tonicity of the saline the more complete the sphering of the corpuscles unless some other factor is superimposed, on the basis of osmotic relations alone it might seem that the hemolysis in the incubated series would vary inversely with the tonicity of the saline. However, in the present incubation series hemolysis varied directly with the percent of the saline. The lower incubation hemolysis in 0.41% saline might be thought due to the smaller number of corpuscles present after the first hour because of the initial osmotic hemolysis, or to a possible protective action of products of the initial hemolysis. When the hemolysis was computed for the 0.41% and 0.70% series after 22 hours incubation for all of the 36 samples, on the basis of number of corpuscles present in the 0.41% series after the initial osmotic hemolysis, the mean hemolysis for the 0.70% series was over 17% greater. This value was highly significant by the "t" procedure in spite of the facts that an average of 22% of the corpuscles were eliminated from the 0.41% series by the initial osmotic hemolysis, and that the 0.70% series were approaching total hemolysis after 22 hours incubation. Some subsidiary tests in which hemolysed erythrocytes were added to preparations having no initial hemolysis and others in which the prod-

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ucts of the initial hemolysis were removed from the corpuscles remaining before these were incubated showed that the products of hemolysis had little or no influence on the development of incubation hemolysis in these series. As there was no initial osmotic hemolysis in all of the series including and above 0.70% saline direct comparisons can be made between those series. The possible effect of the phosphate buffer on hemolysis was eliminated by substituting a carbonate buffer in some of the tests. The results were essentially the same with the two buffers. Ham and Castle(1) infer that the increases in erythrocytic volume and fragility during incubation are due to metabolic processes which produce an increase in the osmotically active constituents of the erythrocytes. If this explanation be accepted it would seem that the salines from 0.41% to 1.28% are progressively more favorable to these postulated metabolic changes. The nature of these metabolic changes has not been determined, but Harris(8) followed the losses and gains in potassium and sodium by human erythrocytes with and without dextrose and concluded that metabolic activity was responsible for the movement of these cations.

In the present experiments it was found that 95% or more of the expected hemolysis in 0.70% saline resulting from 22 hours incubation at 37°C could be prevented by the addition of 32 mg of dextrose per 100 ml of the 1:100 saline dilutions of blood, and that as little as 1 mg of dextrose per 100 ml appreciably reduced 22 hour incubation hemolysis. On the other hand, hemolysis could be speeded up greatly, although the relative action of the several salines remained the same, by incubation at 42°C or at 45°C. Both the reaction to dextrose and to higher incubation temperatures might suggest metabolic changes as factors in incubation hemolysis.

Summary. In 1:100 dilutions of dog blood with buffered salines ranging from 0.41% to 1.28% the hemolysis resulting from incubation for 22 hours or less at 37°, 42° and 45°C varied directly although not proportionately with the osmotic tonicity of the saline.

Ninety-five percent or more of the expected hemolysis resulting from incubation in 0.70% saline at 37°C could be prevented by the addition of 32 mg of dextrose to 100 ml of the 1:100 saline dilution of blood, and as little as 1 mg of dextrose per 100 ml appreciably reduced 22 hour incubation hemolysis.

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Effects on Anaphylactic Shock of Salicylates, Aminopyrine and Other Chemically and Pharmacologically Related Compounds.* (17870)

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The effect of salicylates on antibody formation and antigen-antibody reactions has been studied sporadically for many years(1). Recently, Campbell(2) prevented anaphylaxis in a small number of animals by the administration of aspirin. Because salicylates have had a long, useful history in medical therapy, it seemed highly desirable to extend Campbell's results to learn more about the phenomenon and also to search for related

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