cellular infiltration of portal areas and parenchymal fatty metamorphosis; the latter was sometimes periportal in location. These changes continued to be observed in most animals throughout the experimental period with increasing amounts of collagen in portal areas appearing as the experiment progressed. The increase in collagen was definite but never great. In 4 animals thin collagenous trabeculae connected portal areas and surrounded lobules. In a number of other animals fibrous extensions of portal areas were present but incomplete. There was neither prominent lobule distortion nor parenchymal hyperplasia. The failure to produce clear-cut portal cirrhosis is considered to be due to the inconstant and minimal degree of periportal necrosis.

After 9 weeks, the livers showed an increasing incidence of depressed gross lesions of irregular shape and size. The lesions in some livers were of such extent and severity as to produce marked deformity and shrinkage of various lobes. Microscopically, the lesions showed moderate to marked loss of parenchymal cells with collapse of sinusoids and reticulum stroma. In the latter part of the experiment a few such lesions showed marked fibrosis. The probable pathogenesis of these lesions is discussed and the incidence by lobes is given.

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Hemagglutination by Bacterial Suspensions with Special Reference to *Shigella alkalescens.*

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Agglutination of red blood cells by various viruses has interested many workers, and has been found useful in immunological investigations of virus host relationships. Recently, Keogh, *et al.*[1] have reported agglutination of red blood cells by saline extracts of *Hemophilus pertussis, Hemophilus parapertussis,* and *Hemophilus bronchisepticus.* These authors suggest that this property of *Hemophilus pertussis* is related to the virulence of the organism and its ability to produce protective antibodies in mice.

In the course of an incomplete and unsuccessful survey of bacteria for Rh-like antigens using anti-Rh_0 blood typing serum, it was noted that saline suspensions of certain bacteria agglutinated human red cells in the absence of serum. It is the purpose of this report to describe some aspects of this phenomenon.

Experimental Observations. Hemagglutination by various bacterial species. Saline suspensions of bacteria grown for 24 hours on horsemeat infusion agar (and in a few instances on 5% rabbit blood agar) were prepared to have a density equal to 500 p.p.m. silica standard.[2] This type of suspension was used throughout these studies unless otherwise stated. Equal parts of these suspensions and 2% saline suspensions of once washed human Group O Rh-negative red cells were placed in small tubes. Agglutination was looked for after one hour's incubation at 35°C.

No agglutination of human red cells was observed using suspensions of the following bacteria (wherever more than one strain of organism was examined, the number of strains is given in parenthesis): *Aerobacter aerogenes, Alcaligenes fecalis, Bacillus anthracis, Brucella abortus, B. melitensis, Corynebacterium*

[1] Keogh, E. V., North, E. A., and Warburton, M. F., *Nature,* 1947, **160**, 63.

[2] *Standard Methods for Examination of Water and Sewage,* 6th Edition, American Public Health Association, New York, 1925, p. 4.

diphtheriae, C. hektoenii, Diplococcus pneumoniae (2), *Eberthella typhosa* (3), *Escherichia coli, Klebsiella pneumoniae* (2), *Lactobacillus acidophilus, L. bulgaricus, Pasteurella pestis, P. tularensis, Proteus vulgaris* (3), *P. morganii, Pseudomonas aeruginosa, Salmonella ballerup, S. choleraesuis, S. enteritidis, S. paratyphi* (2), *S. schottmulleri, Serratia marcescens, Shigella ambigua, S. dispar, S. dysenteriae* (2), *S. flexneri* (14), *Staphylococcus aureus* (5), *Streptococcus fecalis, S. pyogenes* (1 of 2 strains), *S. salivarius,* and *Vibrio comma* (3 of 10).

Human red cells were agglutinated by *V. comma* (7 of 10), *S. pyogenes* (1 of 2), *Brucella bronchiseptica, Hemophilus pertussis,* and *Shigella alkalescens* (23).

Clumping of red cells by 7 of the strains of *V. comma,* one strain each of *H. pertussis,* and *S. pyogenes* was detectable only on microscopic examination. The suspension of *B. bronchiseptica* gave clumping clearly visible within 5 minutes by gross examination.

The addition of a saline suspension of *S. alkalescens* to human red cells caused clumping of the cells beginning in about 3 minutes. Microscopically, the cell aggregates resembled those produced by the action of isohemagglutinins. The speed of reaction and the degree of clumping of human cells by *S. alkalescens* was greater than that of other organisms studied. Among the bacteria studied, only *B. bronchiseptica* approached this intensity of agglutination. Further observations of the nature of hemagglutination by *S. alkalescens* are therefore presented.

Effect of Shigella alkalescens on red blood cells of various species. As shown in Table I saline suspensions of *S. alkalescens* N.I.H. No. 4 agglutinate the red cells of certain species of animals and do not agglutinate others.

Agglutination of human red cells by *S. alkalescens* No. 4 does not appear to be related to known factors in the red cells, such as blood agglutinogens A, B, O, M or N, and the Rh antigens, since differences would have been expected to occur among this sample of human cells.

Twenty-one strains* of *S. alkalescens* were tested against cells of man, horses, and hogs. All but one strain gave equally strong reactions with human cells; this weakly reacting strain gave definite microscopic agglutination. Against one specimen of hog cells one strain failed to cause clumping. This strain was a different one from that giving the weak reaction with human cells. All specimens gave negative results with a suspension of horse red blood cells.

TABLE I.
Effect on Red Blood Cell of Several Species of Animals by the Addition of Saline Suspensions of *S. alkalescens* N.I.H. No. 4.*

Species	Type	No. of cell specimens	Result
Human	Group O Rh+	115	+
	" O Rh—	22	+
	" A Rh+	112	+
	" A Rh—	17	+
	" B Rh+	30	+
	" B Rh—	3	+
	" AB Rh+	3	+
	" AB Rh—	2	+
Monkey	(*M. rhesus*)	17	+
Hog		8	+
Horse		5	—
Dog		8	—
Cow		15	—
Sheep		2	—
Rabbit	New Zealand	37	—
Guinea pig		25	—
Mice	Swiss	5	—
Hamster		5	—
Rat	Wistar	5	—

* Mixtures of equal parts of 2% red cell suspensions and bacterial suspensions having turbidity equal to 500 p.p.m. silica standard, observed 1 hour at 35°C.

Rh designations refer to the results obtained using an Anti-Rh_0 blood typing serum.

— = no agglutination.

\+ = macroscopic agglutination.

Effect of various factors on the hemagglutinin of Shigella alkalescens. Broth cultures of *S. alkalescens* N.I.H. No. 4 were filtered through a Berkefeld filter, and the clear filtrate gave no reaction with human red cells. The possibility that the hemagglutinin was intimately associated with the bacterial cell was investigated.

A heavy suspension of *S. alkalescens* N.I.H. No. 4 in saline was exposed to sonic vibration at 8,000 oscillations per second for 20 min-

* Obtained from the Bacteriology Department of the U. S. Naval Medical Center, Bethesda, Md.

TABLE II.
Effect of Heat, Phenol, and Formalin on the Ability of *S. alkalescens* N.I.H. No. 4 to Agglutinate Human Red Cells.*

Treatment of suspension of *S. alkalescens* No. 4	Agglutination of human red cells Dilution of bacterial suspension						
	undil.	1:2	1:4	1:8	1:16	1:32	1:64
Fresh suspension	+++	++	++	+	+	±	—
37°C for 2 hr	+++	++	++	+	±	—	—
56 " " 5 min	+++	++	++	+	+	±	—
56 " " 15 min	+++	++	+	+	—	—	—
56 " " 30 "	++	+	+	—	—	—	—
93 " " 1 "	—	—	—	—	—	—	—
0.5% formalin 30 m'n	+++	++	++	+	±	—	—
0.5% phenol 30 min	+++	++	++	+	±	—	—

* Bacterial suspensions equal in turbidity to 500 p.p.m. silica standard. 2% saline suspensions of red cells.
— = no agglutination.
± = microscopic agglutination.
+ = macroscopic agglutination

utes. Supernatant fluid from this suspension when undilute gave only microscopic reactions. Similar heavy suspensions of the organism were then rapidly frozen (in dry ice cellusolve mixture) and thawed 12 times, after which the material was placed in a Sharples centrifuge and spun at approximately 18,000 r.p.m. The supernatant resulting from this treatment gave reactions with human cells when diluted 256 times.

It was noted that human red cells could be washed 5 times with saline without affecting the degree of reaction with *S. alkalescens*. However, suspensions of *S. alkalescens* N.I.H. No. 4 washed with saline showed a progressive loss of hemagglutinin for human cells. Traces of hemagglutinin present after 3 washings disappeared after 6 washings.

These observations indicated that the hemagglutinin was separable from the bacterial cell, and therefore, the factor may have been adsorbed to the filter as previously used. Efforts to wash the material from Berkefeld filters were unsuccessful.

The effect of heat, phenol, and formalin on the agglutination factor of *S. alkalescens* is summarized in Table II.

Heat had an adverse effect on the hemagglutinin, causing slight loss of activity at 37°C for 2 hours, more noticeable effects at 56°C for 15 minutes, and destroying activity in one minute at 93°C. The addition of phenol and formalin to bacterial suspensions appeared to lessen the activity of the hemagglutinin after 30 minutes contact.

Agglutination of human red cells by *S. alkalescens* took place at 5°C and at 56°C as readily as at room temperature.

The production of hemagglutinin by cultures of *S. alkalescens* at 35°C is shown in Table III. Bacterial suspensions of comparable turbidity prepared on various days of growth on agar medium showed that hemagglutinin increased from its level at 2 days to maximum proportions in 5 to 7 days, and thereafter fell in titer.

The possibility that agglutinins for human cells present in various animal serums and tissue juices may have influenced the reactions observed, appeared less probable when *S. alkalescens* grown on a medium containing a pancreatic digest of casein gave equally good reactions.

Antiserums, prepared by injecting formalinized suspensions of *S. alkalescens* into rabbits, agglutinated the organisms. The agglutinated organisms washed free of serum failed to agglutinate human cells.

A phenomenon not well explained was observed in the course of this study. Twenty-three strains of *S. alkalescens* on horsemeat infusion agar were placed in a cold room (5°C) for approximately 6 weeks. At this time, transplants were prepared and from these actively growing organisms suspensions were made, none of which agglutinated human

TABLE III.
Titration of Hemagglutinin in Suspensions (Turbidity = 500 p.p.m.) of *S. alkalescens* on Different Days of Growth*

	Agglutination of human red cells Dilution of bacterial suspension							
Day of growth	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
2nd	++	++	+	—	—	—	—	—
3	+++	++	++	+	—	—	—	—
5	+++	+++	++	++	++	+	±	—
6	+++	+++	++	++	++	+	±	—
7	+++	++	++	+	+	+	+	—
8	+++	++	++	+	+	±	—	—
9	+++	++	+	±				

* Bacterial suspension prepared each day from growth of *S. alkalescens*. Turbidity = 500 p.p.m. silica standard. 2% saline suspensions of red cells.
— = no agglutination.
± = microscopic agglutination.
+ = macroscopic agglutination.

red cells. Additional dried cultures (such as were used originally in these studies) were obtained and fresh transplants from the dried cultures showed agglutinating activity as noted before. Thus it would appear that the hemagglutination factor is labile and under certain conditions may disappear from strains known to have possessed this factor.

Discussion. The phenomenon of hemagglutination by bacteria as reported here appears to be different from reactions reported by Huebner, Thomsen, Friedenreich,[3] and Terada.[4] These authors observed that red cell suspensions in which certain strains of bacteria (*Corynebacterium* and gram-positive cocci) are allowed to grow become agglutinable with all human serums used to test the red cells. Nor does the phenomenon reported here appear to be related to the action of *Corynebacterium hektoenii*,[5] or a mustard bacillus,[6] which impart to certain serums in which they are grown the ability to agglutinate all human and some animal red cells.

The ability of bacterial suspensions to agglutinate red blood cells reported by Kraus and Ludwig[7] in 1902 and later elaborated by Guyot[8] and Fukuhara[9] appears to have received little attention.[10] Kraus and Ludwig reported that certain strains of staphylococci and vibrio agglutinated rabbit cells. They reported that hemagglutination by organisms could be interfered with by the action of specific antiserum. Guyot reported that 12 of 18 strains of organisms designated *B. coli* agglutinated the cells of various animals. Eight of these strains showed considerable difference in the species of animals with which they reacted. He was unable to separate hemagglutinin from the bacterial cell. Fukuhara found that diphtheria, mouse typhoid, paratyphoid, and Friedlander's bacilli and Sarcina contained hemagglutinins for cells of some species of animals. He extracted the hemagglutinin with alcohol from bacterial suspensions treated with 0.1N NaOH. The possibility that such reactions might be used in immunological studies of bacteria, as suggested by Keogh *et al.*, gives bacterial hemagglutinin added significance.

The finding of an active hemagglutinin in cultures of *S. alkalescens* provides a readily grown source of hemagglutinin for the study of the phenomenon of agglutination. In ad-

3 Friedenreich, V., *The Thomsen Hemagglutination Phenomenon*, Copenhagen, 1930.

4 Terada, K., *Taiwan Iyakkai Zasshi*, 1936, **35**, 1267.

5 Davidsohn, I., and Toharsky, B., *J. Inf. Dis.*, 1940, **67**, 25.

6 Grove, E. F., and Crum, M. J., *J. Lab. and Clin. Med.*, 1930, **16**, 259.

7 Kraus, R., and Ludwig, S., *Wien. Klin. Wchnschr.*, 1902, **15**, 120.

8 Guyot, G., *Cbl. f. Bakteriol.*, 1908, **47**, 640.

9 Fukuhara, Y., *Z. f. Immunitätsforsch.*, 1909, **2**, 313.

10 Pearce, R. M., and Winne, C. K., *Am. J. Med. Sci.*, 1904, **128**, 669.

dition, the presence of hemagglutinin may be useful in distinguishing this bacterial species from others of the genus *Shigella*.

The specificity of the action of *S. alkalescens* on human, monkey, and hog red blood cells appears to indicate that some substances common to these three species are present in their red cells, while absent in cells of other animal species. This specificity is in rather marked contrast to the findings of Guyot, and also to the recent report of Keogh.

Summary. 1. Saline suspensions of *S. alkalescens, B. bronchiseptica, H. pertussis, V. comma, S. pyogenes* are capable of clumping human red blood cells.

2. Suspensions of *S. alkalescens* agglutinate human, monkey, and hog red blood cells, and do not agglutinate cells of certain other animal species.

3. The hemagglutinin of *S. alkalescens* can be separated from the bacterial cell by high speed centrifugation after successive freezing and thawing of bacterial suspensions.

4. The hemagglutinin of *S. alkalescens* is labile, being destroyed by heat, and spontaneously disappears from cultures on standing at 5°C for 6 weeks.

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Effect of Thyroxin on Mouse Susceptibility to Polioencephalitis.*

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The fact that a small fraction of persons in a population group display recognizable symptoms of poliomyelitis even in epidemic situations has led to the suggestion that there may be physiological factors involved in determining the susceptibility to the disease.

The higher morbidity rate among children and pregnant women and the possibility of predisposing factors such as physical exertion and preceding infections suggest that this hypothetical physiological factor may be of an unspecific character. An increased metabolic rate is common to all these conditions mentioned. However, it may be noted that the observation of general decreases in BMR during the warmer season of the year when most poliomyelitis epidemics occur is not in agreement with the assumption of an increased metabolic rate as a factor increasing the susceptibility to poliomyelitis.

Recent experimental studies[1] on the influence of environmental temperature on the resistance of Swiss white mice to the poliomyelitis virus showed a marked tolerance of these animals to the infection when acclimated to low temperatures. Since thyroxin secretion is increased upon exposure to cold the influence of thyroid treatment on the susceptibility to poliomyelitis was investigated. On a small number of Swiss white mice it could be shown[2] that thyroactive substances were able to prolong the incubation period of poliomyelitis up to 100% whereas thiouracil had the opposite effect.

The effect of the thyroid hormone on the resistance to neurotropic virus disease is of great theoretical and practical importance and therefore further studies on this subject seemed indicated. Our investigations were concerned with the effect of crystalline thyroxin on the susceptibility of mice to the MM strain of mouse polioencephalitis. Thyroxin was chosen instead of other thyroactive extracts or substances because the effect of crystalline thyroxin on the metabolism lends itself in a more accurate way to a standardization of experimental conditions.

The 3-5-weeks-old mice used for these

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Holtman, D. Frank, *Science*, 1946, **103**, 137.

2 Holtman, D. Frank, *Science*, 1946, **104**, 50.