

ment showed that the activity of penicillin lasted only 2 or 3 hours (see Fig. 1), the following experiment was designed to determine the time required for penicillin administered intramuscularly to reach the blood and peritoneal fluid in detectable amounts and the length of time it persisted.

Mice were injected intraperitoneally with 1 ml of sterile, 4% mucin and intramuscularly with 100 Oxford units of penicillin, and sacrificed at stated intervals thereafter. The heart's blood and peritoneal fluid obtained (.25 to 0.5 ml) were added to 1.5 ml of broth, titrated by 5-fold dilution and inoculated with 2×10^7 meningococci. The highest dilutions which inhibited growth showed that penicillin was detectable in the blood within 5 minutes (the first observation) and for about 2 hours thereafter. In the peritoneal fluid penicillin appeared within 5 minutes, rose for $\frac{1}{2}$ hour to a somewhat higher level than in the blood and fell off about the end of the second hour. The penicillin determinations on the blood are subject to the limitations brought out in a later communication⁶ which describes meningococcal action of fresh serum.

Discussion and Summary. It should be remembered that the method of producing experimental meningococcal infection in the mouse involves the use of mucin, without which meningococci, even the most virulent

strains, will not initiate a genuine infection. The method, nevertheless, lends itself to investigation of the action of penicillin *in vivo*. These studies have so far brought out the following points: Penicillin was equally effective if injected intravenously, subcutaneously, or intramuscularly. When given intramuscularly, it was detectable in the heart's blood within 5 minutes and persisted there for about 2 hours. Its diffusion into the peritoneal cavity occurred simultaneously and its concentration there rose to a relatively higher level than in the blood. (Cf. the clinical observations of Rammelkamp and Keefer⁷ on its diffusion out of serous cavities). The penetration of penicillin into the peritoneal cavity may or may not have been affected by the presence of mucin.

The effect of penicillin on the bacterial population in the peritoneal fluid was an immediate decrease in numbers which was more striking if the infection had progressed for 3 hours than if the inoculum had been introduced only one hour before.

Infection with a stock strain of meningococcus, although fully virulent by ordinary standards, was more easily brought under control than infection with strains recently isolated from human cases of meningococcal infection. The latter required repeated doses of penicillin.

⁶ Miller, C. P., and Foster, Alice Zimmerman, *Proc. Soc. Exp. Biol. and Med.*, in press.

⁷ Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

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Effect on Blood Agglutinins of a Polysaccharide Isolated from *Ascaris suum*.

JOSÉ OLIVER-GONZÁLEZ AND EDUARDO MONTILLA. (Introduced by P. Morales Otero.)

*From the Department of Medical Zoology and the Blood Bank, School of Tropical Medicine, San Juan, Puerto Rico.**

The inhibitory effect of a polysaccharide isolated from the pig roundworm, *Ascaris*

suum, on the α and β agglutinins of human

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serums has been described.¹ The investigation described below has been carried out to determine the effect of the ascarid polysaccharide on anti-Rh, anti-M, anti-N, and on cold agglutinins.

Experimental Methods. Three serum samples with anti-Rh agglutinins were used, 2 of them obtained from 2 cases of erythroblastosis foetalis. The third was a commercial preparation of guinea pig anti-Rh serum. Enough of the polysaccharide was added to these serums to bring its concentration to 4% and the mixture then incubated for 30 minutes in a water bath at 37°C.[†] One drop of a 2% suspension of known Rh positive and Rh negative cells respectively was added to 2 drops of each of the serums. Each serum was tested twice, one specimen with and the second without the polysaccharide. These mixtures were placed in Wassermann tubes and incubated in a water bath for one hour at 37°C, then centrifuged (500 revolutions per minute) for one minute and examined microscopically.

Analogous mixtures with and without polysaccharide were prepared also with commercial preparations of anti-M and anti-N serums.[‡] One drop of a 2% suspension in saline of known M and N cells was added to 2 drops of each of the "treated" and untreated serums. The suspensions were left for 30 minutes at room temperature and examined microscopically at regular intervals.

In another experiment, a 4% concentration of polysaccharide was also added to 5 human serums with cold agglutinins. To 2 drops of the above mentioned "treated" and untreated serums, already placed in Wassermann tubes, one drop of a 2% suspension of the patient's own red cells was added the tube then stored in the icebox overnight at 6°C and examined microscopically on the following morning.

The serums from 5 different rabbits having cold agglutinins were also treated with the same amount of polysaccharide and similarly tested with A human erythrocytes and with the rabbit's own red cells. Previous to storage

in the icebox, the untreated rabbit serums, plus the red cell suspensions, were incubated at 37°C for 30 minutes so as to exclude the possibility of the presence of α agglutinins. After exposure at 37°C for 30 minutes and at 6°C overnight, these suspensions were examined microscopically.

The addition of polysaccharide from *Ascaris suum* had no inhibitory action on the anti-Rh, anti-M, and anti-N agglutinins whenever these were present in the experiment just described. Agglutination of red cells by the homologous agglutinins was observed at about the same intensity in the serums with the polysaccharide as in the untreated ones (Table I).

The polysaccharide had no effect on the cold agglutinins present in either human or rabbit serums. Agglutination of all erythrocytes was seen in the "untreated" and "treated" human and rabbit serums after exposure to 6°C overnight. The rabbit serums showed no agglutinins against human A and rabbit cells after incubation at 37°C for 30 minutes (Table I).

Discussion and Summary. A 4% concentration of the ascarid polysaccharide added to human serums reduces the α and β agglutinins to a zero titer.¹ The results presented above indicated that the polysaccharide, when added to human serums at the same concentration at which it inhibits the α and β agglutinins, has no effect on anti-Rh, anti-M, anti-N, and on cold agglutinins. Thus, its action may be regarded as specific for α and β agglutinins. The fact that a polysaccharide with similar properties has been isolated from other parasites² suggests that certain infectious agents may have a closer relationship to human isoagglutinogens than is known at present.

The specific action of the ascarid polysaccharide may be used to advantage in the preparation of anti-Rh, anti-M, and anti-N serums that require the absorption of the α and β agglutinins. This is particularly true in human serums with anti-Rh agglutinins, where the α and β agglutinins have to be removed so that the serum could be used to detect Rh-positive or Rh-negative cells.

¹ Oliver-González, J., *J. Inf. Dis.*, in press

[†] This and similar mixtures will be referred to as "treated" serums.

[‡] Obtained from Lederle Laboratories, San Juan, Puerto Rico.

² Oliver-González, J., and Torregrosa, Mercedes, *J. Inf. Dis.*, in press.

TABLE I.
Failure of a Polysaccharide from *Ascaris suum* to Inhibit Anti-Rh, Anti-M, Anti-N, and Cold Agglutinins.

Test materials			Positive agglutination with erythrocytes	
No. and source of serum samples	Agglutinins	Erythrocytes tested	Untreated serums	Serums treated with 4% polysaccharide
3 { 2 human 1 guinea	Anti-Rh	Group O Rh+ Group O Rh-	Rh+	Rh+
1 commercial	Anti-M	M and N	M	M
1 "	Anti-N	M and N	N	N
5 human	Cold agglutinins	Patient's cells	Patient's cells	Patient's cells
5 rabbit	Cold agglutinins, no agglutinins at 37°C	Group A and rabbit's own cells	Group A and rabbit's own cells	Group A and rabbit's own cells

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Poliomyelitis Virus in the Human Oro-pharynx.*

HOWARD A. HOWE, HERBERT A. WENNER,[†] DAVID BODIAN AND KENNETH F. MAXCY.

From Poliomyelitis Research Center, Dept. of Epidemiology, Johns Hopkins University.

In an effort to explain the transmission of poliomyelitis recent investigations have tended to emphasize the finding of virus in the feces. While successful attempts to demonstrate virus in the human nose and throat were made in earlier years these have virtually been abandoned in the face of readily isolated intestinal virus.¹ It is possible, however, that the technical difficulties of virus isolation have played an important role in emphasizing the apparent rarity of virus in the pharynx. The current communication deals with an attempt to vary the methods which have been employed in the past. Instead of the nasal or pharyngeal washing we have employed cotton swabs rubbed against the posterior wall of the oro-pharynx and the peritonsillar area. The swabs were then detached, dropped into a fluid-tight container with 1 cc of sterile water, and stored on dry

ice. An average of 2 swabs was obtained from each subject.

The subjects were patients in the New Haven Hospital admitted during the late summer of 1943. Twenty different specimens were obtained at various times during the first week of the acute illness.[‡] Patients were paralytic or non-paralytic. While it has been impossible to test the entire series of swabs, 14 specimens have been examined to date and it is felt that the results are of sufficient interest to merit a preliminary report at this time.

The technique of inoculation was as follows. It had previously been shown that elution from cotton or mouse cord emulsion containing Lansing virus was most complete at pH 8. On the other hand, mouse inoculation was more successful with the eluate ad-

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[†] National Research Fellow.

[‡] We wish to thank Dr. Robert Ward for valuable assistance in collecting some of this material.

¹ Vignee, A. J., Paul, J. R., and Trask, J. D., *Yale J. Biol. and Med.*, 1938-39, **11**, 15.