fusely. Under these conditions arterial pressure remained above the necessary height for obtaining an arhythmia much longer than was expected from the hemorrhage. The pulsating heart prevented clotting.

Five animals yielded 1 to 3 premature systolic arhythmias following a like number of benzol insufflations at the time the above mentioned coronary arteries were bleeding profusely. A representative record discloses a perfectly regular arhythmia from insufflations in that it is preceded by a moderate rise in blood pressure (16 mm.) and a slowed and strengthened pulse. Every animal which gave the 'insufflation' arhythmia with the heart exposed, produced one from insufflations when the above mentioned coronary vessels were slit. In one animal the arhythmia was obtained after the left interventricular artery and the left ventricular vein had been ligated close to the atrium.

Since the ventricular arhythmia following insufflations of benzol takes place readily under conditions where the blood supply to the ventricles has been greatly reduced and cannot be increased appreciably, when it normally occurs under conditions which should cause a pronounced increase in the coronary intake, suggests that this arhythmia is not contingent on the coronary flow to the ventricles.

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On the Property of Certain Normal Animal Sera to Neutralize the Virus of Poliomyelitis.*

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It has been known for a long time that the sera of children and monkeys, who have suffered from poliomyelitis, are capable of neutralizing the specific virus, although the frequency of this occurrence appears by no means to be as regular as was formerly believed[1] More recently a high percentage of normal adults, giving no history of a previous attack or of contact with the disease, have also been found to possess virucidal substances in their blood.[2] A

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[1] Jungeblut, C. W., and Smith, L. W., *J. Immunol.*, 1932, in press.

number of authors, by indirect inference, have deduced from epidemiological criteria, that this capacity is acquired through exposure to subclinical infections.[3] In contrast to this viewpoint, we[4] have recently presented direct experimental evidence which strongly suggests that resistance to poliomyelitis as expressed by the fall of morbidity and the rising level of serological activity with increasing age, is predominantly a function of normal physiological maturation and to a large extent seems to develop independently of previous contact with the specific antigen. In further support of such a conception—or rather in opposition to the general validity of the exposure theory—the following observations are reported which bear on the presence of virucidal substances in the normal sera of certain susceptible and non-susceptible animals.

Rhesus monkey. There is general agreement in the literature that the serum of *immature* monkeys is uniformly devoid of neutralizing power. We can confirm this point from a summary of 16 tests on a corresponding number of individual samples of serum obtained from normal rhesus monkeys ranging in probable age between 1½ and 3 years. In no case did we observe any neutralization effect with any of the described sera when amounts of 0.6 to 0.8 cc. of serum were tested against 0.6 cc. or 0.4 cc. respectively of a 10% virus suspension (contact 1½ hours at 37°C., overnight icebox).

Quite different results, however, were obtained when normal sera of *adult* rhesus monkeys were studied. These animals were mature or submature when received, judging not only from size but from development of the teeth and sexual organs. The males carried the testicles in the scrotum and the females were passing, more or less regularly, through definite cycles of menstruation. They were kept in separate quarters belonging to the Department of Anatomy and were never in contact with the infected animals nor the attendants handling them. We had an opportunity to test the serum of seven of such animals, one male and six females, for neutralizing power against poliomyelitis virus. Two of these animals were bled twice, so that the samples under investigation numbered nine in all. Four of these nine samples were found to neutralize the virus *in vitro* perfectly, the remaining five exerted no demonstrable virucidal ac-

[2] Shaughnessy, A. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 463. Aycock, W. L., and Kramer, S. D., *J. Prev. Med.*, 1930, **4**, 189 and 201. Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 409. Brodie, M., *J. Bact.*, 1932, **23**, 102.

[3] Aycock, W. L., *J. Am. Med. Assn.*, 1931, **97**, 1199.

[4] Jungeblut, C. W., and Engle, E. T., N. Y. Academy of Medicine, meeting section of pediatrics, Nov. 12, 1931.

tion in the quantities tested. The four neutralizing sera came from four different female animals and were all obtained within 10 days *after* onset of menstrual bleeding. Two of these animals when bled again a few days *before* onset of the next menstrual cycle gave sera which failed to neutralize. We do not know whether this fluctuation in virucidal power of the serum in the same animal is causally related to the various phases of menstruation or has been due to other factors. Work is under way to investigate more precisely the above mentioned possibility in the human. (Table I.)

TABLE I.

Tests for virucidal power of normal adult Rhesus sera.

No.	Sex	Menstruation	Quantities of Serum cc.	Quantities of Virus cc.	Result of Neutralization Test	Control Monkey Paralysis
I	♀	after	0.6	0.6	D76 No paralysis	D57 Complete, 6 days
II	♀	before	0.6	0.6	D87 Partial par., 12 dys.	D78 Almost comp., 12 dys
III	♀	after	0.6	0.6	D53 No paralysis	D57 Complete, 6 days
		before	0.6	0.6	D96 Comp. par., 7 days	D91 " 11 "
IV	♂		0.6	0.6	D97 Comp. par., 9 days	D91 " 11 "
V	♀	after	0.8	0.4	E30 No paralysis	E32 " 12 "
		before	0.8	0.4	E51 Comp. par., 7 days	E48 " 11 "
VI	♀	irreg.	0.8	0.4	E52 Comp. par., 6 days	E48 " " "
VII	♀	after	0.8	0.4	E53 No paralysis	E48 " " "

Technique: serum-virus mixtures incubated 1½ hr. at 37°C. and kept overnight in icebox. 1 cc. of mixture injected intracerebrally.

Cebus monkey. The South American ringtail, or Cebus monkey, is insusceptible to intracerebral inoculation with poliomyelitis virus as has been observed before by Flexner[5] and by Kraus and Kantor.[6] The mechanism of this natural resistance, however, has remained obscure. In this connection it is interesting to point out that the Cebus monkey does not show evidence of a menstrual cycle or periodic bleeding similar to the macaque or human beings. We have attempted to infect four Cebus monkeys by intracerebral inoculation with poliomyelitis virus without producing any symptoms of the disease. Moreover, the animals remained refractory to repeated inoculation after they had been subjected to splenectomy and bilateral castration. Normal sera obtained from these monkeys before inoculation were tested for neutralizing power in the usual manner. Of the 4 sera three neutralized the poliomyelitis virus. The one serum which failed to neutralize was not retested, however the animal proved equally insusceptible to intracerebral inoculation. (Table II.)

[5] Flexner, J., and Lewis, P. A., *J. Am. Med. Assoc.*, 1910, **54**, 45.

[6] Kraus, R., and Kantor, L., *Rev. Inst. Bact.*, Buenos Aires, 1917, **1**, 43.

TABLE II.
Tests for virucidal power of normal Cebus sera.

No.	Sex	Quantities of serum cc.	Quantities of virus cc.	Result of Neutralization Test			Control Monkey			
I	♂	0.6	0.6	Monkey	D98	No paralysis	D 91	Comp. paralysis,	11	days
II	♂	0.6	0.6	"	E 7	" "	D100	" "	8	"
III	♂	0.6	0.6	"	E50	" "	E 48	" "	11	"
IV	♀	0.6	0.6	"	E49	Comp. par., 9 days	E 48	" "	11	"

Technique: same as given for Table I.

Sheep. Neutralizing substances against poliomyelitis virus in normal sheep sera have been encountered occasionally by certain authors (Flexner and Lewis, Stewart and Hasselbauer[7]) while others (Howitt[8]) have recorded only negative results. We have tested a total of four normal sera obtained from as many different adult sheep at various stages of the oestrus cycle.† None of these sera were capable of neutralizing the virus *in vitro,* even when doses as high as 0.8 cc. of serum were run against 0.4 cc. of virus.

Cock. One sample of serum from a cock was tested for neutralization of the virus *in vitro.* The serum failed to neutralize.

The results obtained in this study indicate that absence or presence of the power to neutralize poliomyelitis virus is unevenly distributed over a number of naturally insusceptible animals. As may be gleaned from the literature, a similar irregularity has been noted with normal sera from other insusceptible animals, like the horse, rabbit, and goat, which sometimes neutralize the virus and sometimes fail to do so. Contrasting the previously mentioned animals with the only known species susceptible to experimental infection except the anthropoid apes, *i. e.,* the rhesus monkey, it would seem that neutralizing substances may occasionally be found in the serum of adult animals, while the serum of immature animals reacts uniformly negatively. The close analogy appears obvious between this occurrence and the development of virucidal power with puberty in man. It permits of only one conclusion, in our opinion, namely that normal neutralizing substances, experimentally behaving identically with immune neutralizing substances may occur under conditions which in the present state of our knowledge preclude previous contact with the specific antigen. The relationship of these normal viru-

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.,* 1910, **55**, 662. Stewart, F. W., and Hasselbauer, P., *J. Exp. Med.,* 1928, **48**, 449.

[8] Howitt, B. F., *J. Inf. Dis.,* 1932, **50**, 26.

† These sera were obtained through the courtesy of Dr. F. F. McKenzie of the Missouri Agricultural College, to whom we express our best thanks.

cidal substances to those arising after recovery or active immunization is unsettled and work is in progress to elucidate that point. In this respect the problem is in no way different from the well recognized occurrence in man and animals of other normal 'antibodies' directed against a variety of infectious and non-infectious antigens; and the vexing question as to the precise origin of these agencies in either case remains unsolved, except for the purely empirical observation that physiological maturation is evidently an important governing factor in their development.