proximately 90% of the nitrogen of both samples was accounted for by the analyses. The light fraction contained 1.14% calcium while the heavy fraction contained 6.39%.

Discussion. The data in this paper show that the calcific changes previously observed to occur with age in the medial elastic tissue of the human aorta(2) are accompanied by changes in the amino acid composition of the elastin. It is doubtful that the shift in amino acid distribution represents a change with time in the amino acid composition of a pure protein. The elastin studied may be a mixture of two or more proteins, and the changes observed may be related to differences in the ratios of the separate constituents How such a change can come about is not yet known. It is possible that the proteins laid down later in life may be different from those formed earlier because they are formed under the influence of fibroblasts which themselves have undergone age changes. Previous reports(3) from this laboratory have emphasized the fact that the pulmonary artery is characterized by a low rate of calcification of the medial elastic elements. This investigation brings forth evidence that no significant amino acid changes occur with age in pulmonary elastin. On the other hand, the high rate of the elasto-calcinosis of the aorta can be correlated with a significant increase in dicarboxylic amino-acids.

It is tempting to speculate that the increased calcium binding capacity of the elastin from the older specimens is somehow related to the increased content of free carboxyl groups. Two mechanisms by which these changes could be related suggest themselves. It is possible that the calcium ion is bound by the free carboxyl groups of aspartic and glutamic acids and is then precipitated as calcium phosphate or some other phosphate-containing calcium complex because of the smaller dissociation constants of the inorganic complexes. The carboxyl group could then again become available for the binding of more calcium. Another possibility is that the calcium is bound by the free carboxyl groups, leaving one valence available for combining with phosphate, which could then bind more calcium, etc. No direct stoichiometric relationship exists between the free carboxyl groups and the calcium, the latter being found in great molar excess per unit weight of the old aortic elastin.

Summary. Comparative analyses of elastin from young and old aortas reveal a pattern of characteristic age changes consisting of increase in specific gravity, calcium, and free carboxyl groups. There is an underlying shift in relative amino acid composition. In contrast to the aorta, the pulmonary artery shows minimal age changes.

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Fixed Rabies Virus in Blood Following Intracerebral Inoculation in Mice and Rabbits. (18605)

DOROTHY H. WONG AND JULES FREUND.

From the Division of Applied Immunology, The Public Health Research Institute of the City of New York.

There is little information on the distribution of viruses injected into the brain of animals. Schaeffer and Muckenfuss(1) studied the distribution of particulate matter introduced in the brain of monkeys by injecting India ink. They found only a very small part of the injected material at the site of its deposition. Schlesinger(2) observed that only 3 to 10% of the estimated amount of virus was recoverable from the infected brains of mice one hour after intracerebral inoculation of the Western equine encephalitis virus or

1. Schaeffer, M. and Muckenfuss, R. S., *Experimental Poliomyelitis*, 1940, p. 119, Sci. Press Printing Co., Pa.

2. Schlesinger, R. W., *J. Exp. Med.*, 1949, v89, 491.

the *E. coli* phage T1 or T7. He made no attempt to demonstrate the virus elsewhere. It was recently reported by Cairns(3) (after the completion of our observation) that C16 bacteriophage active against *Sh. flexner* and the W.S. strain of influenza virus can be demonstrated in the blood of mice within one minute after intracerebral inoculation. This is in harmony with the finding of M. Smith (4) who has shown that virus enters the blood stream within the first 2 hours after intracerebral inoculation of the poliomyelitis virus in mice.

From the point of view of the pathogenesis of rabies infection and the understanding of the test for immunity by intracerebral challenge, it is desirable to know whether the rabies virus behaves like those studied by Cairns and Smith. Since the rabies virus is larger than the viruses found to enter the blood stream following intracerebral inoculation, it seemed desirable to carry out similar experiments with rabies virus.

Materials and methods. Swiss white mice weighing from 8 to 10 g or rabbits weighing about 1500 g were used. Two strains of fixed rabies virus were employed, one received from the National Institute of Health, Bethesda, Md., and the other from the Illinois Department of Health. The LD_{50} of both strains was approximately 10^{-6}. One gram of mouse brain containing fixed rabies virus was suspended in 9 ml of distilled water containing 2% horse serum and 20 units of penicillin per 0.03 ml. The suspension was centrifuged at 1000 RPM for 10 minutes and the supernate used for injection in 3 groups of mice. Two other groups were injected with a 1:1000 dilution of the brain suspension. Mice were injected in one hemisphere of the brain receiving 0.03 ml of the virus suspension. Rabbits were similarly injected with 0.1 ml of the 1:10 brain suspension. To facilitate the collection of blood from mice heparin was injected subcutaneously in 4 groups of mice as indicated in Table I. At various intervals of time following the intracerebral inoculation, blood was collected aseptically either directly from the heart or by clipping the heart of the mouse. From the rabbits the blood was obtained either from the ear vein, the external or internal jugular vein, or the heart. The blood was centrifuged at 2000 RPM for 10 minutes. 0.03 ml of serum or plasma was injected intracerebrally into recipient mice. Suspensions of brains of recipient mice which developed symptoms of rabies were passed into new mice. Only when the latter mice died of rabies was a blood specimen considered positive. All mice were observed for a period of 3 weeks.

A total of 22 specimens of blood from 5 rabbits, taken during the first 3 hours following intracerebral inoculation, was tested; 12 from the ear vein, 4 from the external jugular vein, 4 from the internal jugular vein and 2 from the heart. One specimen of spinal fluid obtained 2 hours and 40 minutes after injection was tested.

Results. Table I gives the results of the 5 experiments in which the mouse was used. In all 5 experiments using mice, virus was demonstrated in the blood plasma or serum at some time during the first 2½ hours following intracerebral injection of the fixed rabies virus. There was no specific period of time during the 2½ hours in which every blood specimen tested for that period gave positive results. The blood serum or plasma of 15 host mice transmitted rabies to recipient mice; that of 24 mice failed to produce rabies. Only 2 blood specimens caused rabies in all recipient mice injected with an aliquot of that specimen; 13 transmitted the disease to one or more but not all of the recipient mice.

When rabbits were used as the host animal rabies virus was demonstrated in one of 5 rabbits. This was the specimen taken from the ear vein 3 hours after the intracerebral injection of the virus and produced rabies in one of 4 recipient mice receiving 0.03 ml of plasma. The one specimen of spinal fluid did not produce rabies in the recipient mice.

Discussion. The mechanism by which the virus reaches the blood stream is not known. It is possible that it enters the blood stream through blood vessels damaged by the injec-

3. Cairns, H. F., *Nature,* 1950, v166, 910.

4. Smith, M., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 88.

TABLE I. Fixed Rabies Virus in the Blood of Mice Following Intracerebral Injection.

	Host mouse				Blood recipient mouse	
	Rabies virus					
Exp.	Strain	Dilution	Heparin subcut., ml	Bleeding time interval after intracer. inoc.	No. inj.	No. rabies positive
1	I.D.P.H.	10^{-1}	0.1	2 min.	5	0
				15 ''	5	0
				30 ''	3	0
				40 ''	5	1
				1 hr	4	0
				1 '', 15 ''	5	0
				1 '', 20 ''	5	0
				2 ''	3	1
				2 '', 5 ''	2	0
				3 ''	4	0
				3 '', 5 ''	6	0
2	N.I.H.	10^{-1}	0.1	15 ''	3	0
				30 ''	3	3
				45 ''	2	1
				1 hr	3	1
				1 '', 30 ''*	7	0
				2 ''	4	2
				2 '', 30 ''	4	2
3	N.I.H.	10^{-3}	0.1	30 ''	3	0
				45 ''	4	0
				1 hr	2	0
				1 '', 30 min.	3	0
				2 ''	5	0
				2 '', 30 ''	2	1
4	N.I.H.	10^{-3}	0.1	15 ''	5	0
				30 ''	5	1
				45 ''	4	1
				1 hr	2	0
				1 '', 30 min.	5	0
				2 ''	1	1
				2 '', 30 ''	7	1
5	N.I.H.	10^{-1}	—	15 ''	5	1
				30 ''	5	2
				45 ''	4	0
				1 hr	4	1
				1 '', 30 ''	4	0
				2 ''	5	0
				2 '', 30 ''	6	0

* 2 mice were used.

tion, but it seems more probable that it reaches the blood stream via the cerebrospinal fluid. The latter possibility is supported by the findings of Schaeffer and Muckenfuss(1) on the distribution of India ink following intracerebral injection in monkeys. They found "the distribution of the material following intracerebral deposition varied to some extent. In most cases, however, little of the material remained at the site of inoculation, but rapidly entered the cerebrospinal fluid either by seeping backward through the path of inoculation into the subarachnoid space or via the ventricles into the spinal canal." Insofar as the final deposition of the inoculum is concerned, they could draw no definite conclusion(5). It may be assumed that the rabies virus having reached the cerebrospinal fluid passes through the arachnoid villi into the blood stream. It is stated by Best and Taylor(6), however, that true solutions and colloids pass through the arachnoid villi but particulate matter does not. If we are correct in our assumption, it is of interest that a virus as large as the fixed rabies virus, which

5. Schaeffer, M. and Muckenfuss, R. S., *Am. J. Path.*, 1938, v14, 227.

6. Best, C. H., and Taylor, N. B., *The Physiological Basis of Medical Practice*, 1950, p. 1069, Williams and Wilkins Co., Baltimore.

has been estimated to be 0.1 to 0.21 micron (7-9) in size, can pass through the arachnoid villi.

At the outset of the experiments we had expected that after intracerebral injection the virus would be readily demonstrable in the blood of the rabbit, in blood samples taken from the internal jugular vein. This assumption was not confirmed.

Summary. Fixed rabies virus was recovered from the blood stream of the mouse and rabbit during the first 3 hours following intracerebral inoculation.

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Metabolism of Radioactive Vitamin B_{12} by the Rat.* (18606)

KENDALL W. BARBEE AND B. CONNOR JOHNSON.

From the Division of Animal Nutrition, University of Illinois, Urbana.

Radioactive vit. B_{12} labeled with Co^{60} has been prepared by Rosenblum *et al.*(1) by fermentation. We have used this compound† to study the metabolism of vit. B_{12} in the rat. Chow *et al.*(2) have reported that approximately 80% of the vitamin is excreted in the urine within 24 hours when injected subcutaneously, while at least 270 γ/300 g rat is needed to cause the excretion of the vitamin in the urine when administered orally.

In the present experiment we have studied the retention and excretion of radioactive vit. B_{12} when given orally and when given by injection and also the form of the Co (whether as vit. B_{12} or other) stored and excreted.

Experimental. Four adult white rats were used for this experiment. Rats A and B were used for qualitative work, and rats C and D for quantitative work. One ml of Co^{60} labeled vit. B_{12} solution containing approximately 20 γ of vit. B_{12}, approximately 1.3 microcuries of radioactivity, was administered to each rat. The vit. B_{12} was injected subcutaneously into rats A and C and was given orally with the dry ration of ground checkers fed to rats B and D. The excreta were collected at 24-hour intervals for 3 days, at which time the animals were sacrificed. Paper strip chromatograms were run on the urine, feces, and extracts of the kidneys, liver and spleen of rats A and B. The solvent system was that used by Winsten and Eigen(3). Radioautographs were made of these chromatograms and of a pure radio-vit. B_{12} chromatogram. A paper strip chromatogram was also

TABLE I. Radioautographs of Paper Strips.

	R_f (of spot)
Cobalt chloride	.0
Vit. B_{12}	.05
Vit. B_{12b}	.08 to 0.13
Rat A (given B_{12} by inj.)	
24-hr urine	.13 (B_{12b})
Kidney	.095 (B_{12b})
24-hr feces	.0 (Co^{60++})
Liver	No spot
Rat B (given B_{12} orally)	
24-hr urine	.07 to 0.10 (B_{12b})
24-hr feces	0.0; .074 (Co^{60++} and B_{12})
Kidneys, Liver, Spleen	No spots

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2. Chow, B. F., Barrows, L., and Lang, C., *J. Nutrition*, 1950, v42, 405.

3. Winsten, W. A., and Eigen, E., *J. Biol. Chem.*, 1949, v181, 109.