THE REMOVAL OF CALCIUM FROM THE BLOOD BY DIALYSIS IN THE STUDY OF TETANY.*

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On the appearance of the paper by Abel, Rowntree, and Turner[1] describing a method by which diffusible substances might be removed from the circulating blood by dialysis, it occurred to us that we might attack the question of the relation of calcium salts to tetany in a new way.

At first we thought that we might be able to produce tetany by mechanically removing calcium from the blood by allowing it to circulate through celloidin tubes immersed in an isotonic solution containing no calcium. We therefore constructed a machine somewhat like Abel's, and made a number of experiments with this object in view, using a fluid put together according to Abderhalden's analysis of the inorganic constituents of the dog's blood, but without calcium. We varied this fluid by substituting phosphates for chlorides, with the idea that by removing chlorides also we might still further reduce the proportion of soluble calcium. We used defibrinated blood instead of salt solution in starting the current, and finally defibrinated all the animal's blood. The results were practically negative, and we abandoned the method because it seemed that we could attain our end better in another way without exposing the animal to an operation which must be prolonged over many hours.

We adopted a method which consisted in passing a quantity of defibrinated blood from another dog through a modified dialyzing apparatus (text-figure 1) for many hours until, as we could ascer-

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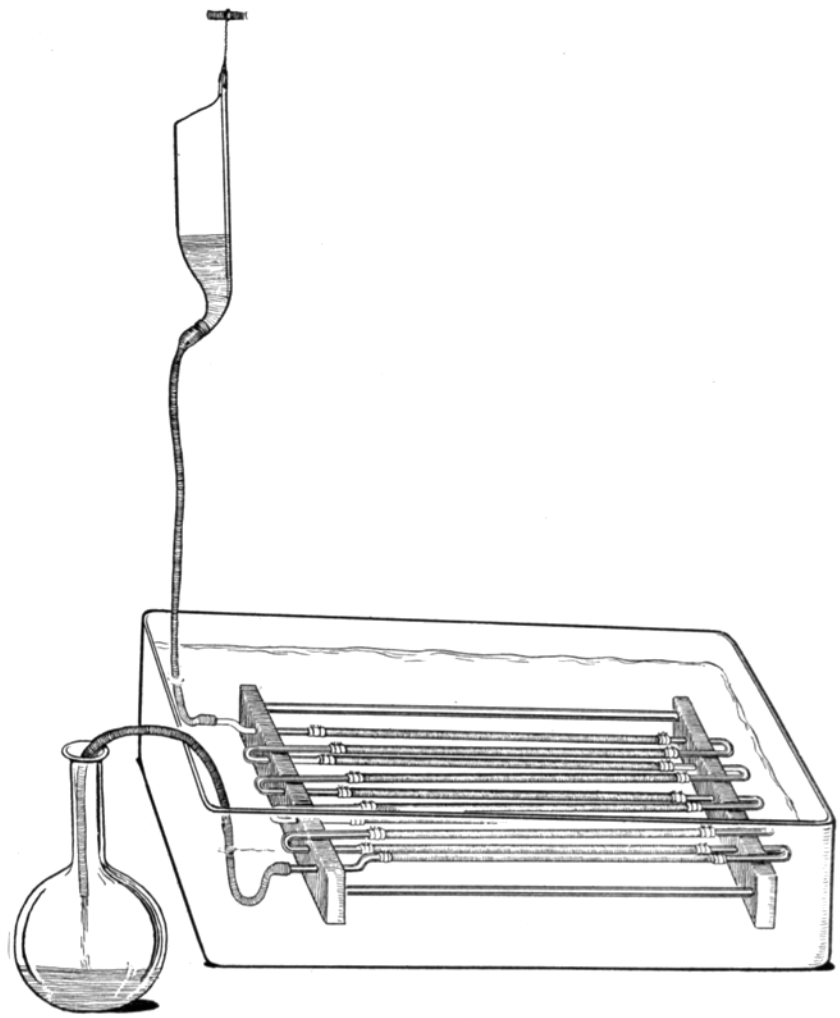
[1] Abel, J. J., Rowntree, L. G., and Turner, B. B., *Jour. Pharmacol. and Exper. Therap.*, 1914, v, 275.

tain by chemical analysis, the amount of calcium was greatly reduced and had passed over into the dialyzing fluid. It was then



TEXT-FIG. 1. Dialysis apparatus of celloidin tubes in series.

proposed to run the blood into the veins of a normal animal after an equal amount had been removed from the carotid artery, but even before this was done, it was thought best to ascertain the effect of the dialyzed blood upon the nerves of an isolated extremity by perfusion, according to the method previously employed[2] in studying the blood of animals in tetany. A number of experiments made in this way gave the following results.

Dog 1,420.—Normal extremity. Perfusion started at 3.50 P. M. with blood dialyzed against calcium-free fluid.

	2.00	3.30	3.50	4.00	4.10	4.20	4.40	4.50
KC[3]	0.2	0.05	Perfusion started	0.05	0.05	0.05	0.1	0.2
KO	—[4]	—		0.6	1.8	1.2	1.6	2.4
AC	0.5	1.0		0.2	0.5	0.6	0.4	1.2
AO	1.4	0.4		0.5	0.5	0.6	0.4	1.2

Blood used in perfusion contained calcium per 1,000 c.c. as follows:

Before dialysis.	After dialysis.	After perfusion.
0.085	0.0465	0.080

Dog 1,421.—Normal isolated extremity. Perfusion started at 4.15 P. M. in the same way.

	3.50	4.00	4.15	4.20	4.30	4.45	5.00	5.15	5.30
KC	0.4	0.6	Perfusion	0.2	0.4	0.4	0.4	0.4	0.4
KO	—	—		1.6	2.8	1.8	1.8	2.6	1.6
AC	1.0	1.6		1.2	1.0	1.0	1.2	1.0	1.0
AO	1.2	2.0		1.2	1.0	1.0	1.0	0.8	1.0

Dog 1,427.—Normal leg perfused first with normal then with dialyzed blood.

	8.45	9.00	9.30	9.45	9.50	10.00	10.10	10.15	10.20	10.30	10.40	10.50	11.00	11.10	11.20	11.30	11.35	11.50
KC	0.3	0.4	Perfusion complete Normal blood	0.6	0.8	0.8	1.0	1.0	0.6	0.6	0.6	0.6	0.8	0.8	1.0	1.0	2.0	1.2
KO	—	—		—	—	—	—	—	4.4	3.6	4.0	3.4	3.4	—	—	—	—	—
AC	1.6	1.6		2.4	2.4	2.4	2.8	2.4	2.0	2.0	2.0	2.0	2.2	2.2	2.4	3.4	3.4	4.0
AO	1.8	2.4		—	4.6	4.6	3.2	2.6	2.0	2.0	2.0	2.0	2.0	1.4	2.4	2.2	2.6	4.0

Dialyzed blood Normal blood

[2] MacCallum, W. G., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv, 941.

[3] KC = kathode closing; AO = anode opening, etc.

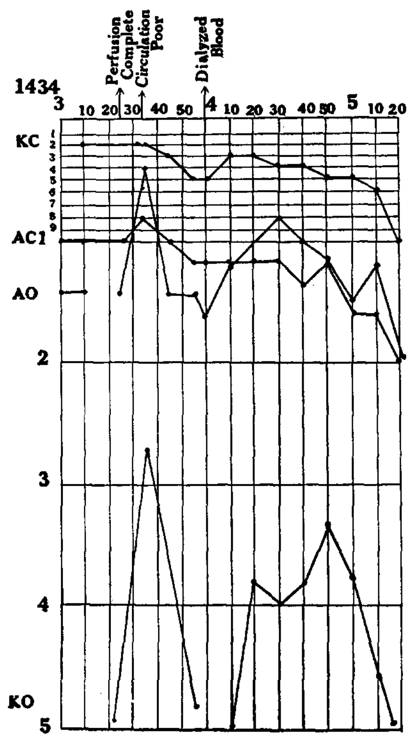
[4] In all the tables — indicates negative up to 5 milliamperes.

It will be seen from these tables that the results of perfusion with dialyzed blood were not especially striking, nor do they convince one that the alterations in excitability of the nerves are strictly due to the withdrawal of calcium. From long previous experience, although it is known that a rise in excitability of a brief and temporary nature can come about from inadequacy of the circulation and that the great fall at the end of each curve is doubtless due to this or to some direct injury of other kinds, still it is also known that with a good circulation with normal blood the excitability will remain almost constant. These curves were especially inconclusive in that while a visible contraction could be elicited with weak currents, strong shocks gave only about the same muscular jerk. Instead of a brisk sharp contraction the foot moved lazily, often twisting in a peculiar way, and after the perfusion with dialyzed blood had gone on for only a short time all peripheral contractions disappeared and the shock produced only jerking of the thigh muscles behind the electrode. These are inevitable signs of approaching death of the nerve, impressing us rather as the consequences of some sort of poisoning, and in every case the dialyzed blood was quickly exchanged for normal blood, sometimes with a return of the excitability.

Discouraged by these results, we determined to add the normal amount of calcium to our dialyzing fluid, which at this time had the following composition:

Sodium chloride	60.0
Disodium phosphate	28.5
Potassium chloride	4.0
Magnesium chloride	2.87
Sodium bicarbonate	5.0
Dextrose	10.9
Water	10,000

It became apparent at once, as we might have foreseen, that the calcium is precipitated by the phosphates present. Phosphates were therefore left out and calcium was added. After dialysis with this fluid, perfusion of the leg gave almost the curve which one would expect with normal blood.



TEXT-FIG. 2. Perfusion of leg with blood dialyzed against fluid containing all inorganic constituents of blood including calcium.

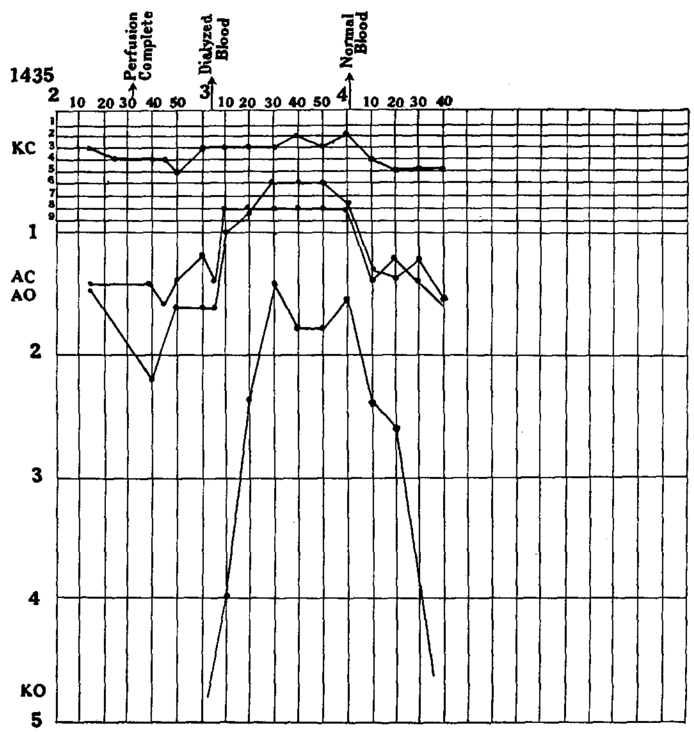
Dog 1,434.—(Text-figure 2.) Normal leg perfused with blood dialyzed against a fluid containing calcium but no phosphates.

Sodium chloride	60.0
Sodium bicarbonate	5.0
Potassium chloride	4.0
Magnesium chloride	2.3
Dextrose	11.0
Calcium lactate	6.0
Water	10,000

	3.10	3.25	3.35	3.45	3.55	4.00	4.10	4.20	4.30	4.40	4.50	5.00	5.10	5.20
KC .	0.2	Perfusion	0.2	0.3	0.5	0.5	0.3	0.3	0.4	0.4	0.5	0.5	0.6	1.0
KO .	—		2.8	—	—	—	5.0	3.8	4.0	3.8	3.4	3.8	4.6	6.0
AC .	1.0		0.8	1.0	1.2	1.2	1.2	1.2	1.2	1.4	1.2	1.6	1.6	2.0
AO .	1.4		0.4	1.4	1.4	1.6	1.2	1.0	0.8	1.0	1.2	1.6	1.2	2.0

It will be seen from text-figure 2 that during the perfusion of normal blood a partial clamping off of the supply tube produced a brief rise in excitability which then passed away when the current was turned on in its full strength.

Therefore, from that point the experiments were begun again with a dialyzing fluid containing neither calcium nor any considerable quantity of the disturbing phosphates. Probably with this fluid the blood lost some of its phosphates, but the difficulty of devising a dialyzing fluid which should remove nothing but calcium from the blood seemed insurmountable.[5]



TEXT-FIG. 3. Perfusion of leg with blood dialyzed against fluid devoid of calcium.

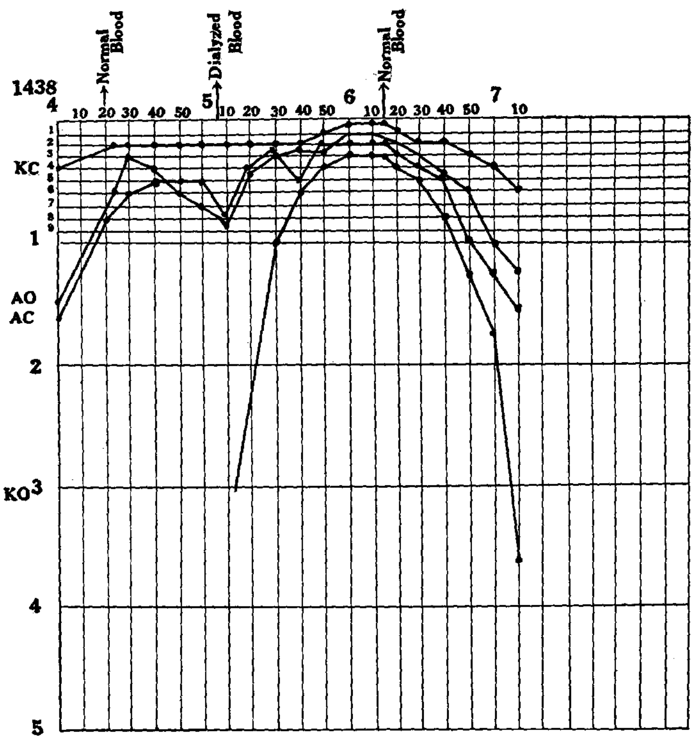
Dog 1,435.—(Text-figure 3.) Normal leg perfused at 2.32 P. M. with normal blood, at 3.01 with dialyzed blood, and at 4.01 again with normal blood. 900 c.c. of normal blood were dialyzed for 7½ hours against 18,000 c.c. of fluid (fluid 3) of the following composition, which was used in all later experiments.

[5] Since writing this paper it has been suggested to us by Dr. A. E. Taylor that as the phosphates in the blood are in non-dialyzable combinations there is little source of error in using a dialyzing fluid without phosphates.

Sodium chloride	60.0
Sodium bicarbonate	5.0
Magnesium chloride	2.30
Potassium chloride	4.0
Dextrose	10.9
Water	10,000

	2.15	2.18	2.32	2.40	2.45	2.50	3.00	3.05	3.10	3.20	3.30	3.40	3.50	4.00	4.10	4.20	4.30	4.40
KC	0.3	0.4	Perfusion complete	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.4	0.5	0.5	0.5
KO	—	—		—	—	—	—	—	4.0	2.4	1.4	1.8	1.8	1.6	2.4	2.6	—	4.0
AC	1.1	0.8		1.4	1.6	1.4	1.2	1.4	0.8	0.8	0.8	0.8	0.8	0.8	1.4	1.2	1.4	1.6
AO	1.4	1.0		2.2	2.0	1.6	1.6	1.6	1.0	0.8	0.6	0.6	0.6	0.8	1.4	1.4	1.2	1.6

Dialyzed blood (at 3.05) Normal blood (at 4.10)



TEXT-FIG. 4. Perfusion of leg with blood dialyzed against fluid devoid of calcium.

Dog 1,438.—(Text-figure 4.) Normal leg perfused at 4.20 P. M. with normal blood. This was changed to dialyzed blood at 5.02, and again to normal blood at 6.12. The dialyzed blood was 1,000 c.c. which had been poured through the long celloidin tubules for 7 hours against 24,000 c.c. of the fluid described above.

	4.00	4.20	4.25	4.30	4.40	4.50	5.00	5.05	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.40	6.50	7.00	7.10
KC..	0.4	Perfusion complete	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.05	0.05	0.1	0.2	0.2	0.3	0.4	0.6
KO..	—		..	—	—	—	—	—	—	—	1.0	0.6	0.4	0.5	0.3	0.4	0.5	0.8	1.2	1.8	3.6
AC..	1.4		0.4	0.6	0.5	0.5	0.5	0.8	0.8	0.4	0.2	0.5	0.2	0.2	0.2	0.3	0.4	0.5	1.0	1.2	1.4
AO..	1.4		0.6	0.3	0.4	0.6	0.7	0.8	0.8	0.4	0.2	0.2	0.2	0.1	0.1	0.2	0.3	0.3	0.6	1.0	1.2

Normal blood (4.25) Dialyzed blood (5.05) Normal blood (6.20)

On the entrance of the dialyzed blood the leg jerked and moved itself about. Twitchings were evident until about 5.20.

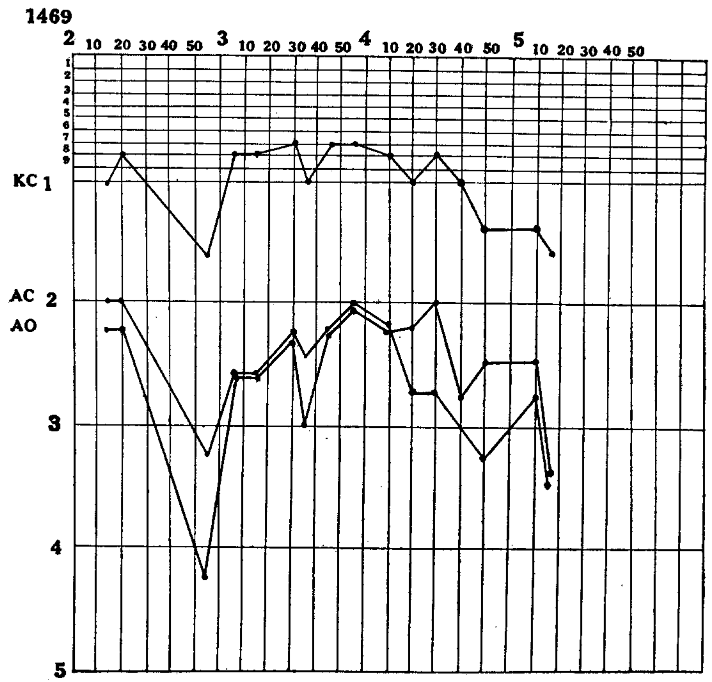
The chemical analysis of the dialyzing fluid before and after dialysis, and of the blood at various points in the experiments, is as follows. The figures show the amount of calcium per 1,000 c.c.

	Dog 1,435.	Dog 1,438.
Dialyzing fluid before dialysis of blood	0.0040	0.0043
Dialyzing fluid after dialysis of blood (1st)	0.0076	0.0076
Dialyzing fluid after dialysis of blood (2d)	0.0048	0.0054
Blood before dialysis	0.0364	0.0442
Blood after dialysis	0.0284	0.0233
Blood after perfusion of leg	0.0212(?)	0.0300

Text-figures 3 and 4 show clearly that perfusion with blood partly deprived of its calcium by dialysis produces great hyperexcitability of the nerves, and further that this must be due solely to the loss of calcium, since exactly the same procedure, changed only by the addition of precisely enough calcium to the dialyzing fluid to prevent any loss or gain of calcium by the blood, brings about no such hyperexcitability of the nerves. This proof may be somewhat strengthened by the addition of another curve made in the same way, except that the dialyzing fluid was so devised as to remove a large part of the chlorides from the blood, leaving the calcium unchanged.

Dog 1,469.—(Text-figure 5.) Normal leg perfused at 2.45 P. M. with normal blood, and at 3.30 with dialyzed blood. Dialysis was of 800 c.c. of normal blood for 6½ hours against 30,000 c.c. of fluid 4 of the following composition:

Sodium acetate	50.0
Sodium nitrate	40.0
Sodium bicarbonate	10.0
Magnesium chloride	2.3
Potassium nitrate	5.25
Calcium lactate	5.0
Dextrose	20.0
Water	10,000



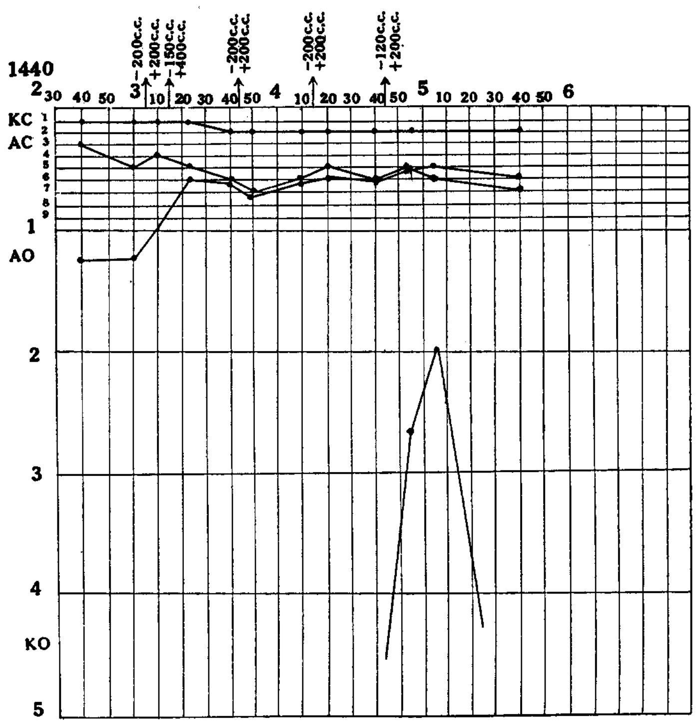
TEXT-FIG. 5. Leg perfused with blood dialyzed against a chloride-free fluid.

	2.15	2.20	2.45		2.55	3.05	3.15	3.30	3.35	3.43	3.55	4.10	4.20	4.30	4.40	4.50	5.10	5.15
KC...	1.0	0.8	Perfusion	Normal blood	1.6	0.8	0.8	0.7	1.0	0.7	0.7	0.8	1.0	0.8	1.0	1.4	1.4	1.6
KO...	—	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—
AC...	2.0	2.0			3.2	2.6	2.6	2.2	2.4	2.2	2.0	2.2	2.8	2.8	3.0	3.2	2.8	3.4
AO...	2.2	2.2			4.2	2.6	2.6	2.2	3.0	2.2	2.0	2.2	2.2	2.0	2.8	2.6	2.6	3.4

Dialyzed blood

It is seen from text-figure 5, in which the variations are so slight, that hyperexcitability of the nerves is not produced by long perfusion, even with blood greatly altered by dialysis, so long as the dialysis has not removed the calcium. When the calcium is diminished, however, the effect in producing hyperexcitability is prompt and persists as in the case of tetany blood so long as blood altered in this way is passing, giving way to normal conditions as soon as normal blood is again circulated.

When an isolated extremity is perfused the influence of the undiluted dialyzed blood must be felt by the nerves, since there is but little tissue from which calcium or other constituents could be absorbed in the passage of the blood. We thought, therefore, that this might be the most trustworthy expression of the nature of the influence of the dialyzed blood. Nevertheless we attempted to introduce dialyzed blood in quantity into the veins of an animal, realizing that it would at once meet with an abundant source of calcium in the tissues, but hoping to circulate it so rapidly as to surprise the nervous system and produce some hyperexcitability not only of the peripheral nerves but of the ganglion cells. We planned not only to wash out all the animal's blood with dialyzed blood but to replace that, too, with fresh dialyzed blood. Indeed after one or two experiments we even circulated fresh dialyzed blood through the whole animal, so that if the tissues were to supply calcium to all the new blood presented to them they must furnish enough to bring back to normal the calcium content of five or six times as much blood as was possessed by the animal before. At the beginning of each



TEXT-FIG. 6. Repeated bleeding from carotid with replacement of blood dialyzed until poor in calcium.

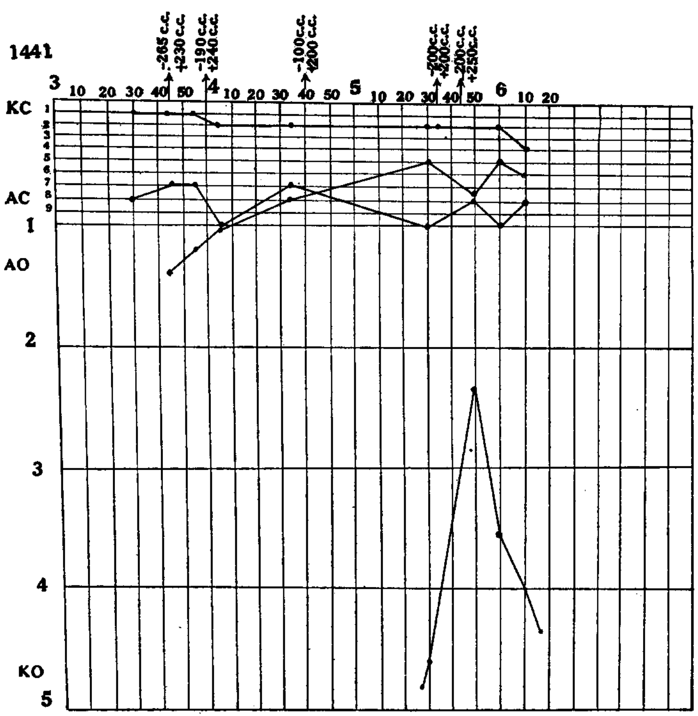
experiment the parathyroids of the animal were removed. The protocols are as follows:

Dog 1,440.—(Text-figure 6.) Normal animal bled at intervals, dialyzed blood being returned. Thyroids and parathyroids removed at 4.35 P. M. No tetany developed.

	2.40	3.00	3.10	3.25	3.40	3.50	4.10	4.20	4.45	4.55	5.05	5.40
KC.	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
KO.	—	—	—	—	—	—	—	—	—	2.6	2.0	—
AC.	0.3	0.5	0.4	0.5	0.6	0.7	0.6	0.5	0.6	0.5	0.6	0.7
AO.	1.2	1.2	1.0	0.6	0.6	0.7	0.6	0.6	0.6	0.5	0.5	0.6

Bled	200 c.c.	150 c.c.	200 c.c.	120 c.c.
Returned dialyzed blood	200 c.c.	400 c.c.	200 c.c.	200 c.c.

In this experiment 1,350 c.c. of blood dialyzed for 7 hours against 30,000 c.c. of fluid 3 were used in replacing normal blood. Effect very slight.



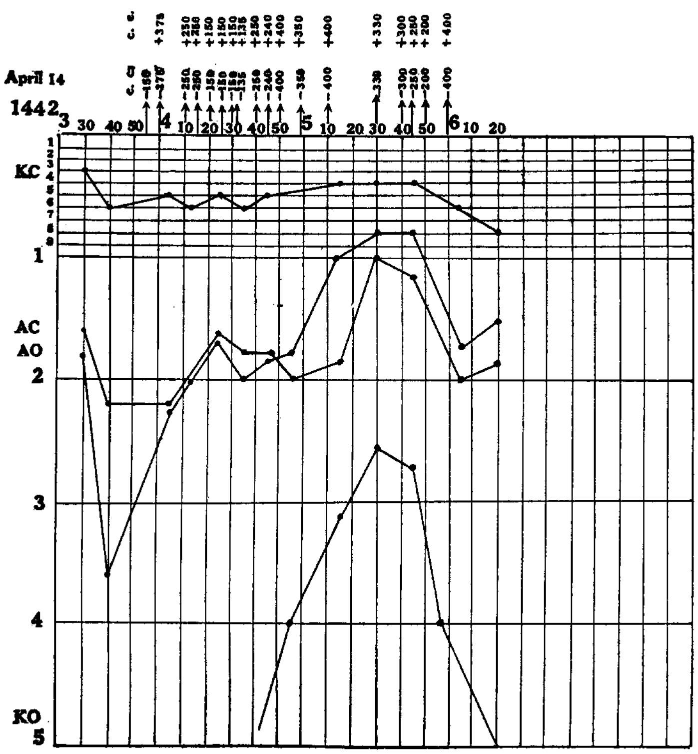
TEXT-FIG. 7. Normal animal. Blood replaced at intervals with dialyzed blood.

Dog 1,441.—(Text-figure 7.) Normal animal, blood at intervals replaced with dialyzed blood. 1,300 c.c. of blood dialyzed for 7½ hours against 26,000 c.c. of fluid 3. Parathyroids were removed just before the experiment started.

	3.30	3.45	3.55	4.05	4.35	5.30	5.50	6.00	6.10
KC .	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.4
KO .	—	—	—	—	—	4.6	2.4	3.6	4.0
AC .	0.8	0.7	0.7	1.0	0.7	1.0	0.8	1.0	0.8
AO .	1.4	1.4	1.2	1.0	0.8	0.5	0.8	0.5	0.6

Bled	265 c.c.	190 c.c.	200 c.c.	200 c.c.
Returned dialyzed blood	230 c.c.	240 c.c.	200 c.c.	250 c.c.

There is general twitching; no definite tetany; much vomiting.



TEXT-FIG. 8. Normal animal through which dialyzed blood was actually circulated.

Dog 1,442.—(Text-figure 8.) Normal animal through whose blood vessels dialyzed blood was actually circulated, as shown in the chart. Parathyroids first removed.

	3.20	3.40	4.05	4.12	4.25	4.35	4.45	4.55	5.15	5.30	5.45	6.05	6.20
KC...	0.3	0.6	0.5	0.6	0.5	0.6	0.5	0.5	0.4	0.4	0.4	0.6	0.8
KO...	—	—	—	—	—	—	—	4.0	3.2	2.6	2.8	4.0	5.0
AC...	1.6	2.2	2.2	2.0	1.6	1.8	1.8	2.0	1.8	1.0	1.2	2.0	1.8
AO...	1.8	3.6	2.2	2.0	1.6	2.0	1.8	1.8	1.0	0.8	0.8	1.8	1.6

Bled	150 c.c.	275 c.c.	250 c.c.	250 c.c.	150 c.c.	150 c.c.	135 c.c.	250 c.c.	240 c.c.	400 c.c.	350 c.c.	400 c.c.	330 c.c.	300 c.c.	250 c.c.	200 c.c.	400 c.c.
Returned dialyzed blood	375 c.c.		250 c.c.	250 c.c.	150 c.c.	150 c.c.	135 c.c.	250 c.c.	240 c.c.	400 c.c.	350 c.c.	400 c.c.	330 c.c.	200 c.c.	250 c.c.	200 c.c.	400 c.c.

1,800 c.c. of blood dialyzed for 7 hours against 30,000 c.c. of fluid 3 were used. Each part of this probably circulated two and one half times. Vomiting; no definite twitching; collapse and death.

From these experiments it seems clear that it is impossible in a short time to reduce the calcium content of the blood circulating through the whole body or to keep it reduced to such a degree as to bring about a tetany-like hyperexcitability of the nerves. There is too abundant a source for fresh calcium in the various tissues, including the bones, and the blood quickly replenishes itself. It might seem possible to repeat this process during several days and thus imitate the latent period which follows extirpation of the parathyroids, but in this case it would be necessary to extirpate those glands, when tetany would arise of itself, since otherwise the blood would rapidly replenish its calcium, under their influence, from the skeleton. Indeed, the rapidity with which calcium enters the circulating dialyzed blood from the tissues is shown by comparison of the analyses of samples taken before and after its circulation in the body.

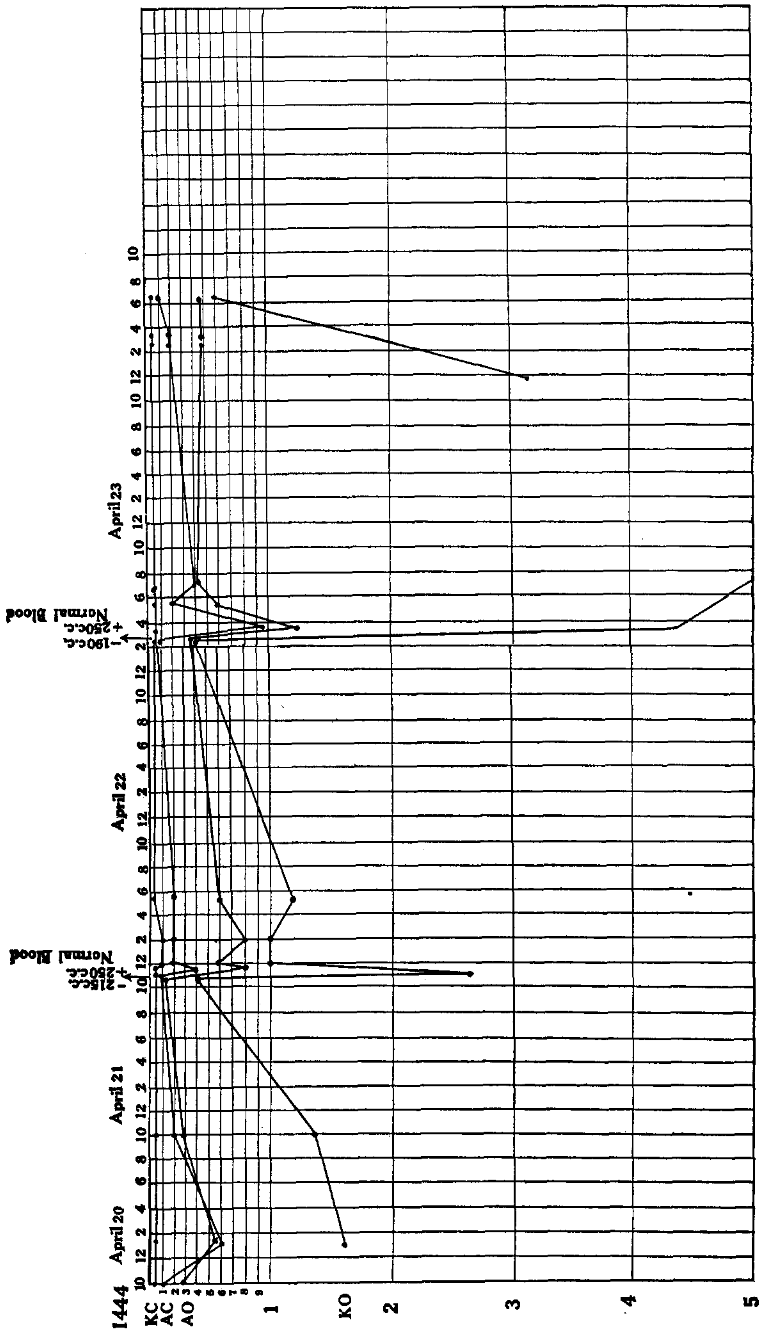
In dog 1,441 the blood contained per 1,000 c.c.:

Before dialysis	0.0485
After dialysis	0.0190
After circulation for about 2 hours	0.0381

It remains a question whether, if one could continue this depletion over several days, it would be possible to produce such changes in excitability as are seen in tetany, and in fact this was our original problem. It leaves one with the impression that the parathyroid secretion must govern the conversion of a non-dialyzable and useless combination of calcium into a dialyzable form which is essential for the control of the excitability of the nerves, but which is lost in the excreta and not newly formed in the absence of parathyroid secretion.

Although we had learned that it is possible by perfusion with calcium-poor blood to produce hyperexcitability of the nerve, it remained to bring this fact into direct relation with the condition in tetany, for it might be objected that there the mechanism is a different one. This we thought would be attained if we could show that this dialyzed blood had lost the power possessed by normal blood of curing tetany on transfusion. It is well known that if an animal in tetany, after the removal of the parathyroids, be bled and the blood replaced with normal blood, the excitability of the nerves falls, and twitchings disappear for a time. Dialyzed blood poor in calcium has little or nothing of this effect, although if the dialyzed blood be not so poor in calcium as that of the animal in tetany, it will lower the excitability somewhat. If in an animal in tetany we replace the blood with dialyzed blood and find no change in the symptoms nor lowering of the excitability, we may remove that blood and replace it with normal blood which does stop the twitching and lower the excitability.

But if, although the excitability is not lowered by the dose of dialyzed blood, we do nothing further, the excitability is found to be lowered the next day or after some hours. To explain this we have imagined that while in dialysis we remove the calcium from the normal blood, we may not remove the parathyroid secretion which on being injected into a dog in tetany after a time causes the appearance of dialyzable calcium in the blood and goes far to relieve temporarily the tetany. It is on this basis that it seems hopeless to attempt to produce a condition resembling tetany by the repeated replacement of the blood over a period of days with normal blood dialyzed until it is poor in calcium, because at the same time we furnish parathyroid secretion which ensures the continual withdrawal of calcium from the tissues. It is true that we might dialyze tetany blood, but even then having a blood free from parathyroid secretion and poor in calcium we have no animal in which to make evident the effect of pure lack of calcium, for the animal with intact parathyroids will continually frustrate this object, and the animal deprived of parathyroids will develop tetany during the experiment.

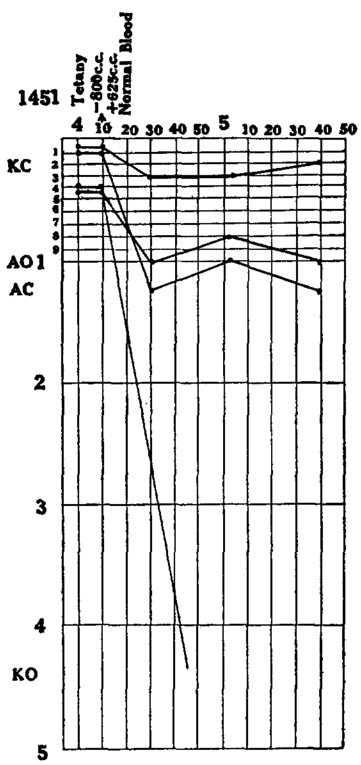


TEXT-FIG. 9. Tetany. Bled on two occasions; normal blood replaced. Transient effect.

ILLUSTRATIVE PROTOCOLS.

Dog 1,444.—(Text-figure 9.) Tetany developing 2 days after parathyroidectomy. Bled on two occasions, normal blood being replaced. Transient relaxation and lowering of excitability.

	April 20.		April 21.						April 22.				April 23.		
	10.00	1.45	10.00	11.15	11.40	12.00	2.00	5.45	2.30	2.55	5.30	6.35	2.20	3.10	6.30
KC	0.05	0.05	0.05	0.05	0.05	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO	.....	1.6	1.4	0.4	2.6	1.0	1.0	1.2	0.4	4.4	—	—	—	—	0.6
AC	0.1	0.6	0.2	0.05	0.4	0.2	0.2	0.2	0.05	1.0	0.2	0.4	0.2	0.2	0.1
AO	0.3	0.6	0.3	0.1	0.8	0.6	0.8	0.6	0.4	1.2	0.6	0.4	0.5	0.5	0.5
Bled					215 c.c.					190 c.c.					
Returned normal blood					250 c.c.					250 c.c.					



TEXT-FIG. 10. Tetany. Effect of bleeding and replacing normal blood.

Dog 1,451.—(Text-figure 10.) Violent tetany 24 hours after parathyroidectomy. Bled 800 c.c., and this loss was replaced by 625 c.c. of normal blood. Immediate relaxation and lowering of excitability of nerves.[6]

[6] A curious phenomenon was observed in these cases in which nearly all the

	April 26.					April 27.	April 28.
	4.00	5.00	5.20	5.55	6.30	11.00	12.15
KC.......	0.05	Blood replaced by normal	0.3	0.3	0.2	0.1	0.05
KO.......	0.4		—	—	—	—	0.3
AC.......	0.1		1.2	1.0	1.2	1.2	0.05
AO.......	0.4		1.0	0.8	1.0	1.2	0.2
Bled		800 c.c.					425 c.c.
Returned normal blood		625 c.c.				Returned dialyzed blood	400 c.c.

This animal developed tetany again on April 28 and was bled 425 c.c., 400 c.c. of dialyzed blood being replaced. There was no recovery from the spasm brought on by the bleeding, and death followed rapidly. This case forms an example of the contrast between the effects of introducing normal and dialyzed blood.

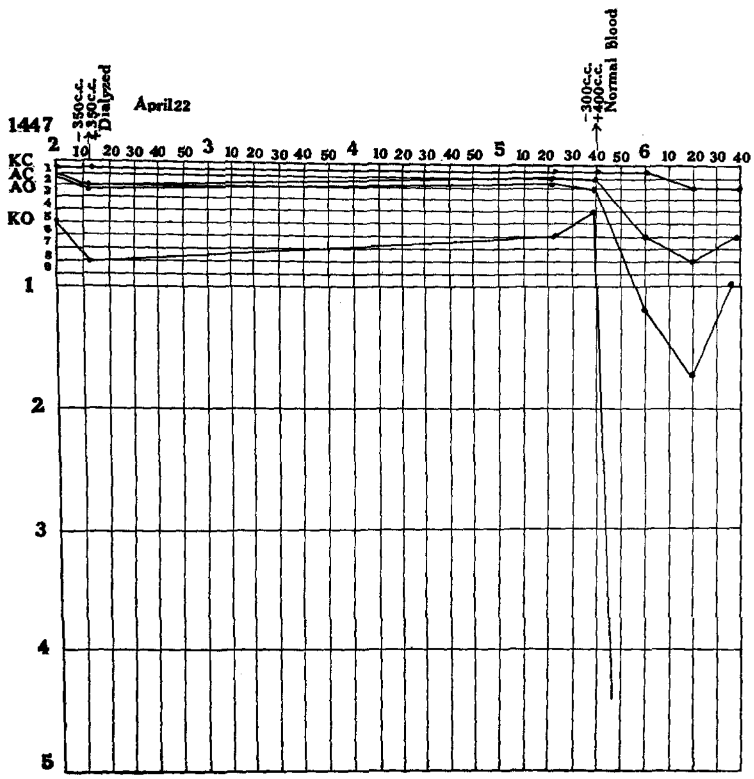
Dog 1,445.—Moderate tetany 2 days after parathyroidectomy. At 2.23 P. M. bled 150 c.c. Blood replaced by 150 c.c. of blood dialyzed for 5 hours against 10,000 c.c. of fluid 3. At 3.20 bled 150 c.c., and replaced 150 c.c. of normal blood. At 10.30 bled 200 c.c., and replaced 250 c.c. of normal blood.

	April 20.									April 21.						
	1.30	1.45	2.20	2.30	2.40	3.00	3.20	3.45	4.25	10.00	10.45	11.00	11.15	12.00	2.00	5.45
KC.	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO.	0.2	0.3	0.3	0.3	0.4	0.3	0.6	1.4	0.6	0.2	0.4	0.4	0.4	—[7]	2.0	1.4
AC.	0.05	0.1	0.05	0.05	0.05	0.05	0.1	0.2	0.1	0.05	0.05	0.1	0.1	0.1	0.2	0.3
AO.	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.5	0.3	0.3	0.4	0.5	0.4	0.4	0.3	0.3
Bled			150 c.c.				150 c.c.			200 c.c.						
Returned dialyzed blood			150 c.c.	Returned normal blood			150 c.c.			200 c.c.						

Dog 1,447.—(Text-figure 11). Violent tetany at 2 P. M. Bled 350 c.c. which were replaced with 350 c.c. of blood from a lot of 600 c.c. which had been dialyzed for 4½ hours against 12,000 c.c. of fluid 3. Change in character of electric reaction, but no marked change in excitability. Contraction slow, no rigidity. Tongue tremor and twitchings continue. At 5.45 bled 300 c.c., and replaced 400 c.c. of normal blood. Complete relaxation and return of excitability to normal.

blood was removed, at the moment before the normal blood was allowed to run in. Although twitching may have been slight when the bleeding began, the most violent contractions and extreme rigidity appeared during the extreme anemia, to disappear at once when the other blood entered the veins. Probably this is due to temporary asphyxia of the nerves.

[7] Negative up to 3 milliamperes.



TEXT-FIG. 11. Tetany. Bleeding on two occasions. Dialyzed blood replaced without effect. Normal blood reduces excitability.

	April 22.										
	11.45	2.00	2.15	2.55	5.25	5.40	6.00	6.20	6.45	9.00	12.00
KC..	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.2	0.2	Quiet; no tongue tremor	Twitching; tremor in tongue.
KO..	0.5	0.5	0.8	Tongue and muscle tremor	0.6	0.4	—	—	—		
AC..	0.1	0.1	0.2		0.1	0.1	0.6	0.8	0.6		
AO..	0.1	0.1	0.2		0.1	0.2	1.2	1.8	1.0		

Bled 350 c.c. 300 c.c.
Returned dialyzed blood 350 c.c. Returned normal blood 400 c.c.

Dog 1,452.—No tetany 4 days after parathyroidectomy. Moderately high excitability. Bled 315 c.c., replaced by 330 c.c. of blood dialyzed 7 hours against fluid 3, $\frac{430 \text{ c.c. blood}}{10{,}000 \text{ c.c. fluid}}$. Animal much depressed and vomited. Excitability a little lowered, and this lowering continued and increased for the next two days. Then bled 300 c.c., which were replaced by 300 c.c. of blood dialyzed for 6 hours, $\frac{400 \text{ c.c.}}{15{,}000 \text{ c.c.}}$. No marked change in excitability.

	April 29.					May 1.				
	5.00	5.25	5.35	5.50	6.05	2.00	5.00	5.40	5.50	6.25
KC..........	0.05	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1
KO..........	1.8	2.6	2.0	2.0	2.0	—	—	—	—	—
AC..........	0.4	0.8	0.8	1.2	1.0	1.4	1.2	1.2	1.2	1.2
AO..........	0.5	0.8	0.8	1.0	0.8	2.0	1.4	1.4	1.6	2.0
Bled		315 c.c.					300 c.c.			
Returned dialyzed blood		330 c.c.					300 c.c.			

We made a number of analyses of the blood of animals in tetany, of the dialyzed normal blood used to replace their own, of the normal blood, and of the normal or dialyzed blood removed again after it had circulated for a time in the animal, thinking that a comparison of these figures might throw some light on what occurred in the body to influence the tetany. But such figures would be difficult to interpret because they would change continually with the time the new blood was allowed to stay in the body, first through the process of obtaining a balance with the tissues, and then through the same process that disturbed the composition of the blood to produce tetany. It might be expected then that thoroughly dialyzed blood left in a tetany animal would become richer in calcium for awhile, although normal blood introduced in the same way would steadily lose calcium until it became very poor indeed, when another attack of tetany might come on, as in dog 1,451.

If tetany blood be dialyzed under exactly the same conditions as normal blood, it still loses a proportionate amount of its calcium, which would perhaps show that it is not especially the loss of a diffusible calcium as contrasted with a non-diffusible form which is important in producing tetany. The blood of dog 1,451 taken during tetany contained 0.0287 of a gram of calcium per 1,000 cubic centimeters. 500 cubic centimeters dialyzed for 5 hours against 12,000 cubic centimeters of fluid 3 then contained 0.0114 of a gram per 1,000 cubic centimeters.

CONCLUSIONS.

We may devise a fluid containing practically all the inorganic diffusible constituents of the blood except calcium, and use it to dialyze normal blood in such a way as to remove from it a large part of its calcium. The dialyzed blood when perfused through an isolated extremity produces an extreme hyperexcitability of the nerves quite like that observed in tetany. Since perfusion with blood dialyzed in precisely the same way against a fluid of the same composition, but containing calcium in the proportion found in the normal blood, causes no hyperexcitability of the nerves, it is evident that the hyperexcitability is due to the lack of calcium. This effect can be attained in only a slight degree by replacing the blood of a whole animal with the dialyzed blood, since under the conditions of the experiment the tissues cannot be sufficiently depleted of their calcium. It seems probable that the parathyroid secretion is not removed by dialysis, but is returned to the body with the dialyzed blood.

To bring this result into relation with the condition in tetany following parathyroidectomy, animals in tetany were bled and the blood was replaced in one case with normal blood, in the other with dialyzed blood poor in calcium. The normal blood immediately relieves the tetany and lowers the excitability, while the dialyzed blood does not. We therefore believe that this is a further proof that in the tetany of parathyroidectomy also the twitching and hyperexcitability of the nerves is due to lack of calcium in the blood and tissues.