simply indicate that there is not sufficient end-piece present to act with mid-piece in bringing about hemolysis and not that there is a complete absence of end-piece. It is, of course, apparent that these experiments are only rough titrations of hemolysis.

On the whole, it is indicated by these experiments that probably there is no greater separation of mid-piece from end-piece brought about by the presence of lipoid in the distilled water dilution of the serum than there is without lipoid. If there was greater separation we would have expected a difference in Table II between the two preparations. This did not occur. The uniform toxicity of globulins from normal guinea pig serum (prepared with lipoid) for guinea pigs is not to be explained upon this basis. It is suggested that the character of the precipitate may in some manner be modified and that the resulting solution-suspension of globulins is found in a different "colloidal state" which contributes to the toxicity of the preparation.

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[2] Bordet, J., and McKinley, E. B., *Compt. rend. Soc. biol.*, 1925, t. xcii, 762-764.

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Effect of High Voltage Cathode Rays on Rickets and on the Activation of Cholesterol.

ARTHUR KNUDSON AND W. D. COOLIDGE.*

From the Laboratory of Biological Chemistry, Albany Medical College, and Research Laboratory, General Electric Co., Schenectady, N. Y.

It has been shown repeatedly that ultraviolet radiation brings about healing of rickets and also that irradiation of cholesterol by ultraviolet light renders it antirachitic. Recently, one of the authors[1] has developed a means for producing high voltage cathode rays outside of the generating tube. It seemed interesting to study the effect of these rays on experimental rickets in rats, first when applied directly, and second when used for the activation of cholesterol.

* With the assistance of H. E. Tanis, Jr., and F. S. Randles.

Rats from 4 to 5 weeks old and weighing from 40 to 60 gm. were put on the Steenbock rachitic diet No. 2965 (2) and after four weeks showed advanced rickets, as evidenced by radiographic examination. Various exposures of the rachitic rats to the cathode rays were tried, but in only one case was the slightest evidence of healing observed. These experiments are summarized in Table I.

TABLE I.

Direct Cathode Ray Exposure

No. of rats	Experimental period	Time of each exposure	Distance from window	Milliamperes	Volts	Result shown by radiographic examination	Remarks
	days	seconds	inches				
1	14	0.1	1	1	200,000	No healing	Very sick
1	9	0.1	2	1	"	"	Died on 9th day
1	14	0.1	4	1	"	"	Very sick
1	12	6.0	1	1	"	"	" "
1	12	6.0	1	1	"	Slight healing	" "
2	14	1.0	1	1	"	No healing	" "
3	14	0.4	1	1	"	"	Very sick—1 died on 12th day
2	14	1.6	1	1	"	"	
3	14	1.0	1	1	100,000	"	
2	5	60.0	1	1	"	"	Both died on 5th day
3	14	6.0	1	1	"	"	Very sick
1	5	30.0	1	1	"	"	Died on 5th day
1	13	30.0	1	1	"	"	Died on 13th day
3	14	1.2	1	1	350,000	"	All died on 14th day*
3	14	0.3	1	1	"	"	Two died on 14th day*

* During the experimental period these rats seemed more sensitive to the touch than those rayed at the lower voltages.

The rats were rayed on the abdominal area every day but Sunday, of the experimental period, for the time given in the third column.

The effect of the cathode ray upon the animal tissue was quite marked even with the shortest exposures. The first effect is a brown coloration of the hair. Later there may be falling out of hair, inflammation of the exposed area, with exudate coming from the skin, and scab formation. The largest exposures used, at the different voltages, caused so much damage to the rats, that it clearly would not have been feasible to use appreciably greater dosage.

The conclusion seems justified that, with voltages of 100, 200 and 350 thousand, rickets in rats cannot be cured by direct treatment with cathode rays.

Experiments were also carried out to ascertain whether cholesterol could be endowed with antirachitic potency by exposure to the

cathode rays. One gram portions of cholesterol were dissolved in ether in a petri dish three inches in diameter, and the ether allowed to evaporate. The cholesterol, evenly distributed over inner surface of the petri dish, was then exposed to cathode rays at a distance of 1 inch, at 200,000 volts and 1 milliampere. These experiments are summarized in Table II, and show that cholesterol may be activated by cathode ray treatment.

TABLE II.

Test Material: Cathode Ray Treated Cholesterol

No. of rats	Experimental period	Amount in diet	Cathode ray exposure of cholesterol: 1 ma., 200,000 volts, 1 inch	Result shown by radiographic examination
	days		Time (seconds)	
2	14	0.5 per cent	30	Complete healing
3	14	0.1 per cent	30	Slight healing
3	14	0.2 per cent	30	Advanced healing
5	14	0.3 per cent	30	Complete healing
3	14	0.4 per cent	30	Complete healing
2	14	2 mg. daily†	30	No healing
2	14	4 mg. daily†	30	No healing
3	14	0.3 per cent	0.1	Slight healing
3	14	0.3 per cent	1	Moderate healing

† Equivalent to 0.025 per cent and 0.05 per cent in diet respectively.

TABLE III.

No. of rats	Experimental period	Treatment			Result shown by radiographic examination	Remarks
		Cathode rays (1 in., 1 ma. 200,000 volts) Daily exposure	Cholesterol treated by cathode rays, 30 sec., 1 in., 1 ma. 200,000 vol.	Ultraviolet radiation distance 1 foot Daily exposure		
	days	seconds	per cent	minutes		
2	14	0.1	0.3	none	Complete healing	Exudate—Quite sore
2	14	6.0	0.3	none	" "	" " "
2	14	none	0.3	none	" "	
2	14	0.1	none	1	Nearly complete	Exudate—Quite sore
2	14	6.0	none	1	" "	Exudate—Quite sore
2	14	none	none	1	Complete healing	Very sick

The effect of direct ultraviolet radiation is ascribed to the activation of cholesterol or related compounds that are normally present in the skin. The cathode ray treatment of cholesterol outside of the body has been shown to activate it. One would therefore expect the cholesterol in the skin to be activated by cathode rays, especially

in view of the fact that the experiments of Table III indicate that direct treatment with cathode rays does not interfere with the healing produced by ultraviolet light.

The fact that the direct exposure of rats to cathode rays has not been found to cure rickets is presumably to be explained by the fact that, owing to severe damage done to the animal by the treatment, it is not possible to give the dose which would be required to activate enough of the cholesterol of the skin to bring about the desired result.

Along with these experiments we have also studied in some cases the ash content of dry extracted femurs, inorganic blood phosphorus, and the line test, upon which we will report in detail elsewhere.

[1] Coolidge, W. D., *J. Franklin Institute*, 1926, ccii, 693.

[2] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, lxiv, 263.

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Contaminating Substances as a Factor in the Activation of Cholesterol by Irradiation.

ALFRED F. HESS AND A. WINDAUS.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Chemical Laboratory, University of Goettingen.

In a recent communication[1] we reported the effects of irradiation on a large number of cholesterol derivatives prepared by one of us (Windaus). Among other derivatives which were tested biologically, and which failed to be activated, was a phytosterol extracted from rapeseed oil. It had been expected that this preparation would develop antirachitic properties after subjection to the rays from the mercury-vapor lamp. This failure indicated that activation in general may be due to some contaminating substance rather than to an alteration in the cholesterol itself.

Some previous experiences had suggested a similar interpretation—for example, the slight changes in physical constants which came about in cholesterol following irradiation, and again the fact that activated cholesterol could be fractionated into an active and an inactive fraction by means of liquid ammonia[2]. The ammonia-soluble fraction, representing somewhat less than 4 per cent of the original cholesterol, was found to have antirachitic potency.