

1. That the poisonous principle of the seeds of *Abrus precatorius* (jequirity) is a globulin.*

2. That the activity of this globulin is destroyed by heating its solution to 75° or 80° C., the temperature at which it coagulates.

3. That this globulin produces a remarkable fall of body temperature after subcutaneous injection.

4. That it causes rapidity of breathing shortly before death.

5. That the other actions ascribable to the globulin are: the production of local œdema and inflammation when subcutaneously injected or applied to the eye, the presence, *post-mortem*, of petechiæ beneath the serous membrane, and the occurrence of hæmorrhagic gastro-enteritis.

III. "The Toxic Action of the Albumose from the Seeds of *Abrus precatorius*." By SIDNEY MARTIN, M.D. Lond., British Medical Association Research Scholar, Assistant Physician to the Victoria Park Chest Hospital. (From the Physiological Laboratory, University College, London.) Communicated by E. A. SCHÄFER, F.R.S. Received May 8, 1889.

An account, by Dr. Wolfenden and myself, of the physiological action of the globulin which I extracted from the seeds of the jequirity plant, has been presented to the Royal Society. I have shown† that there are two proteids present in the seeds; a globulin and an albumose. The present paper deals with the physiological action of the albumose.

Dr. Wolfenden and I showed in the paper referred to that the globulin possessed the poisonous qualities of the watery extract of the seeds and of the body called "abrin," described by Drs. Warden and Waddell. After being obtained in the pure state, it produced severe conjunctivitis when applied to the eye, and when subcutaneously injected it caused local œdema and ecchymosis, followed by death with the signs and symptoms of gastro-intestinal irritation and inflammation. It moreover lowered the body-temperature of the pigeon in a remarkable manner. From the method used by Drs. Warden and Waddell in preparing their "abrin," both proteids would be obtained, since they used a watery extract and precipitated the proteids with alcohol. Abrin would, therefore, be a mixture of globulin and albumose. As Dr. Wolfenden and I had found that the

* An account of the physiological action of the albumose of abrus-seeds has been presented to the Royal Society by one of us (M.).—May 10, 1889.

† 'Roy. Soc. Proc.,' vol. 42, p. 331.

globulin is a powerful toxic agent, it was desirable to ascertain whether the albumose possessed the same power and produced the same symptoms.

It is very difficult to obtain the albumose in a pure state separate from the globulin. Boiling the solution, of course, readily precipitates the globulin, leaving the albumose in solution, but as heat destroys the activity of abrus-poison, it cannot be employed in separating the two proteids. Both proteids are also thrown down by saturating their solution with neutral ammonium sulphate. The precipitate thus formed can be redissolved and the solution dialysed, thus removing most of the salt and precipitating the globulin. But I found many objections to this method. The dialysis has to be prolonged over a week, and there is thus great liability to decomposition. Moreover, it is practically impossible to precipitate all the globulin by dialysis, and the ammonium sulphate, traces of which still remain, being itself poisonous, would be likely to vitiate the result in testing the toxic action of the proteid. I therefore abandoned this method and tried the following, which answered perfectly. A concentrated watery extract of the seed was made and filtered direct into an excess of absolute alcohol. The copious precipitate which fell consisted of globulin and albumose. After a few days, the proteids were removed by filtration, washed with alcohol, redissolved in water, and reprecipitated by absolute alcohol. They were allowed to remain under absolute alcohol for several months in order to coagulate the globulin, and were then filtered off, redissolved, and reprecipitated by alcohol, and allowed to remain under alcohol for a few months longer. Altogether some of the proteids were allowed to remain under alcohol for eight months, or longer. At the end of this time they were removed, washed with alcohol, and dried over sulphuric acid. The residue was ground into a yellowish-brown powder, and consisted of coagulated globulin and of unaltered albumose.

For the purpose of inoculation this powder was mixed in distilled water, which had been well boiled to sterilise it and then cooled. The mixture was filtered and the filtrate was clear. It gave the following reactions :—

1. Neutral to test-paper.
2. No precipitate on boiling.
3. Acetic acid gave a precipitate, which mostly redissolved on boiling, coming down again on cooling, and so on. After boiling and cooling, the precipitate was readily soluble in dilute potash, showing that the proteid was not coagulated.
4. Nitric acid caused a precipitate, mostly soluble on heating, coming down again on cooling, &c. This precipitate, like the acetic acid one, is also readily soluble, after being heated, in dilute potash.

5. Copper sulphate gave a precipitate, soluble in excess of the reagent.

6. Copper sulphate and potash gave a "biuret" reaction.

7. Mercuric chloride gave a precipitate, insoluble in excess of the reagent.

These reactions are similar to those already described by me in the paper quoted ('Roy. Soc. Proc.,' vol. 42) with the exception of the behaviour of nitric acid. I stated in my previous paper that nitric acid gave a precipitate in a solution of the albumose, only in the presence of sodium chloride. This still holds true for *dilute* solutions of the albumose; in *strong* solutions, nitric acid gives a precipitate, even if the neutral salt be absent or present in very small quantities.

Fatal Dose of Albumose.—In my earlier experiments I simply weighed the quantity of dry powder to be injected; but this is not so accurate a method as the one I adopted later. About 0.5 gram of the powder was dissolved in sterilised normal saline solution (0.75 per cent.) and filtered. The amount of proteid dissolved in the filtrate was estimated by dropping a measured quantity of the liquid (1 c.c.) into about 30 c.c. of absolute alcohol, which precipitates both the proteid and the small amount of salt in solution. The precipitate and liquid were well boiled together, the precipitate removed, dried at 110° C., and weighed. The weight, deducting the amount of salt, equals the quantity of proteid present in solution. This I found the only really accurate method of estimating the dose of proteid used in inoculation.

In one experiment, 1.3 milligram of albumose was injected under the skin of a rat weighing 197 grams, being a dose of about 6.6 milligrams per kilo. of body weight. The animal was very ill 49½ hours after inoculation, but completely recovered. Double the above dose, viz., 2.6 milligrams, was injected under the skin of a rat weighing 134 grams (19.4 milligrams per kilo. of body weight), but no poisonous symptoms were noticed. A fatal result is, however, noticed if the dose be as large as 60 milligrams per kilo. of body weight; thus a dose of 10 milligrams killed a rat weighing 167 grams within 20 hours.

Symptoms.—The symptoms produced by the albumose closely resemble those noticed when the globulin is hypodermically injected. There is gradually increasing weakness, with rapid breathing, without the occurrence of convulsions or any paralysis.

On the temperature of pigeons the albumose has the same effect as the globulin. In one experiment death was caused in a pigeon by the albumose in 11 hours and 20 minutes after inoculation. The temperature, which at the time of inoculation was 107.6° F., fell in 4 hours and 5 minutes 4.6°, i.e., to 103° F., after which it began to rise.

In another experiment, a pigeon weighing 335 grams was given hypodermically a dose of 20 milligrams albumose, equal to 60 milligrams per kilo. of body weight. In $4\frac{1}{2}$ hours, the animal began to show symptoms of poisoning, and died in about 6 hours or rather longer. As shown in the accompanying chart the temperature began to fall from the first, and with a few rises continued to fall until the animal was nearly dead, when the observations were ceased. The curve of the number of respirations per minute follows very closely the temperature curve, until just before symptoms of poisoning appeared, when, as will be seen in the chart, the respiration curve does not follow the temperature curve.

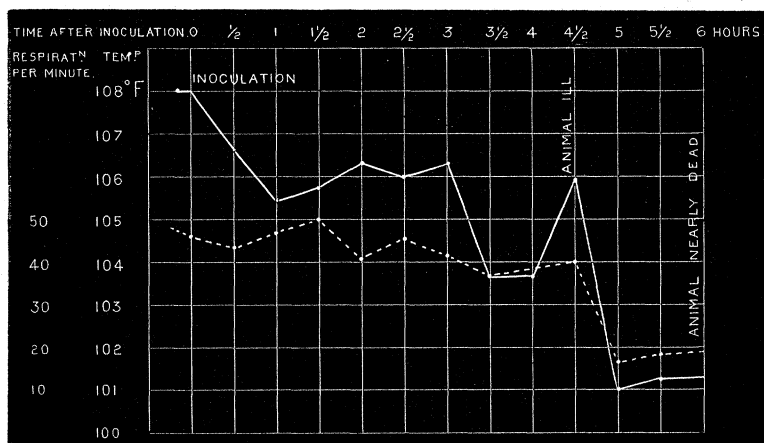


Figure showing effect of Abrus-albumose on temperature and respirations of the pigeon. Temperature taken in rectum every half-hour. Dotted line, respiration; thick line, temperature.

Post-mortem Signs.—These are the same as noticed after death from the globulin. There is local œdema and sometimes ecchymosis, with internally gastro-enteritis, and occasionally petechiæ in the serous membranes.

The blood is in most cases dark and fluid for a long time after death. It may be semi-coagulated.

Effect on the Eye.—The albumose causes severe conjunctivitis when applied to the eye. Thus 1 milligram of albumose dissolved in 2 minims of previously boiled distilled water, instilled into the eye, causes severe conjunctivitis with chemosis in less than 24 hours, and leaves at the end of six days a steamy cornea with leucomata; there are also sub-conjunctival ecchymoses.

Effect of Heat on the Activity of the Albumose.—The poisonous

action of the watery extract of abrus-seed, containing both globulin and albumose, is completely destroyed by boiling the solution. Wolfenden and I have shown in our previous paper that if the solution of the globulin be momentarily raised to 80° C., or between 75° and 80° C., its activity is once and for ever destroyed.

I have tested the behaviour of the albumose in a similar way.

If heated to 50° C. the albumose still retains its power of producing severe conjunctivitis, and of causing death when subcutaneously injected.

If heated to 70°, or 75° C., the albumose is still poisonous, but not to nearly so great a degree as if unheated or heated only to 50° C. If heated to 80° C., this diminishing effect of heat on the activity of the poison is still more seen; so that a solution of albumose containing a lethal dose, and so treated, does not produce poisonous symptoms so soon as an unheated solution, and the animal may recover.

These points are brought out in the following experiments, selected out of many similar ones, all of which were confirmative.

Experiment II. October 13th, 1888.—An equal and lethal dose of albumose injected under the skin of each of three rats. A is unheated; B heated to 75° C.; and C heated to 80° C., previous to inoculation.

A.	B.	C.
White rat, weight 162 grams.	Rat, weight 153 grams.	Rat, weight 108 grams.
Unheated albumose.	Albumose heated to 75° C.	Albumose heated to 80° C.
11 A.M., Oct. 13, 1888. Inoculation made under skin of back.		
2 P.M. No change in any of the animals.		
11 P.M. Animal languid and drowsy. Not easily roused by stimulation. Huddled together. Quite quiet, and not breathing very rapidly.	No change.	No change.
Oct. 14, 1888.		
11 A.M. Found dead.	Found dead.	
P.M.—Rigor mortis well marked. Body cold. Slight œdema at site of injection. No other morbid appearances.	P.M.—Rigor mortis well marked. At site of injection, subcutaneous œdema. Small amount of sticky fluid in peritoneum. No further morbid appearances.	Huddled together. Quite quiet. Breathing rapid. Animal dying. Died during the day.

The above experiment shows the delay of poisonous symptoms produced by heating the albumose up to 75° C. and to 80° C.

In the following experiment, the same fact is brought out, but the result shows recovery from a fatal dose of albumose, heated up to 80° C., and no poisonous symptoms after heating the albumose to 85° C.

Experiment III. November 1st, 1888.—An equal and lethal dose of albumose injected under the skin of three rats:—A is unheated; B is heated to 80° C.; and C to 85° C.:—

A.	B.	C.
White rat, weight 142 grams.	Rat, weight 126 grams.	Rat, weight 125 grams.
Nov. 1, 1888.		
11.20 A.M. Inoculated.	11.10 A.M. Inoculated.	11.15 A.M. Inoculated.
3.30 P.M. No change.	No change.	No change.
Nov. 2, 9.30 A.M. Animal died. Rigor mortis came on immediately.	Animal ill. Quiet, huddled together, and breathing very rapidly. At 12 symptoms became aggravated.	No change.
		No change.
Nov. 3, 10 A.M.	Animal quite recovered. Breathing normal. Takes its food well.	No change. Animal remained quite well.

Similar results were obtained in testing the effect of the albumose in producing conjunctivitis. Raising the solution to a temperature of 75° C. and 80° C. diminishes the effect but does not prevent conjunctivitis developing; the conjunctivitis is less with albumose heated to 80° than with that heated to 75°.

Experiment VI. April 17th, 1889.—One milligram albumose dissolved in 2 minims of distilled water dropped into right and left eye of rabbit. That dropped into left eye was previously heated to 80° C.

<i>Right Eye.</i>	<i>Left Eye.</i>
Unheated albumose.	Albumose heated to 80° C.
April 17, 1889, 10.37 A.M. Inoculation.	10.32 A.M. Inoculation.
„ 18, 10 A.M. Severe conjunctivitis with chemosis.	10 A.M. Very slight inflammation and a little purulent discharge.
„ 23, 10 A.M. Cornea cloudy; with one leucoma. Subconjunctival ecchymosis.	10 A.M. Quite normal.

The conclusions from these experiments may thus be summed up:—

1. *The poisonous activity of abrus-albumose is weakened by momentarily heating its solution to a temperature of 70°, 75°, and 80° C.; and the higher the temperature the greater the diminution.*

2. *The activity of the albumose is completely destroyed by heating its solution up to 85° C.*

This is about five degrees higher than the temperature at which the activity of abrus-globulin is destroyed.

Remarks on the Results obtained.—It is impossible not to be struck with the resemblances in chemical composition between abrus-poison

and the toxic principle of snake-venom. Weir Mitchell and Reichert* have shown that in the American rattlesnakes, the venom contains two poisonous proteids, globulin and a "peptone." The coagulation temperature of the globulin described by them is between 60° and 70° C. The peptone is not a true peptone, as physiological chemists now understand the term; since it is, according to Mitchell and Reichert, precipitated by acetic acid and potassium ferrocyanide. Their peptone, indeed, seems more allied to the albumoses. In the venom of the mocassin, *e.g.*, the "peptone" is precipitated by adding an excess of NaCl to the solution, besides being thrown down by dilute acetic acid. The peptone found by these observers in cobra-venom is precipitated by acetic acid and potassium ferrocyanide, as well as by NaCl added to saturation. A true peptone is not precipitated in this manner, and I cannot but conclude that the body found by Mitchell and Reichert is closely allied to the albumose class of proteids.

The globulin of abrus-seed coagulates between 75° and 80° C. in 10 per cent. magnesium sulphate solution and between 66° and 73° C. in 10 per cent. NaCl solution.† The coagulation temperature in the last solution therefore nearly corresponds to the coagulation temperature of the venom globulin of the rattlesnakes. Mitchell and Reichert do not mention in the presence of which salt the globulin was coagulated. Sodium chloride distinctly lowers the temperature of coagulation.

Abrus-albumose, moreover, closely resembles the "peptones" and "peptone-like" bodies found by the observers in snake-venom. Like them it is uncoagulated by heat,‡ it is precipitated by acetic acid, and by acetic acid and potassium ferrocyanide, and also by saturation with sodium chloride in an acid solution.

Other observers have described in cobra-venom a poisonous albumin and acid albumin (Wolfenden).

Nature of Poison.—I do not propose here to discuss fully the nature of snake-venom. The results of Mitchell and Reichert's researches I would wish to refer to are:—(1) That both the globulin and peptone-like body present in the venom of the rattlesnake are poisonous; (2) that the severe local ecchymosis produced by viperine venom is due to the globulin present in it, since such local effect does not occur after inoculation if the globulin be removed from venom. The third point I would wish to refer to is the effect of heat on the activity of rattlesnake venom. If the venom of the Diamond head (*Crotalus adamanteus*) be heated up to 74° C. the venom is still active; if up to

* 'Researches upon the Venom of Poisonous Serpents.' Philadelphia 1885.

† 'Roy. Soc. Proc.,' vol. 42, p. 333.

‡ Mitchell and Reichert state that cobra-venom may be boiled and filtered; and the filtrate will after a time give a further precipitate on boiling. They explain this by saying a coagulable body is formed from a non-coagulable.

76·5° C. a few symptoms follow a lethal dose, but recovery takes place ; while if heated up to 79·5° C., 81°, and 100°, a lethal dose of the venom does not produce death. The effect of heat varies with different snake-venoms. It takes prolonged boiling to destroy the activity of cobra-venom ; and in some of the American snakes simple boiling does not completely destroy the activity of the venom, although it diminishes it. These results are explicable in the consideration that by coagulation the activity of the globulin is destroyed, and by prolonged boiling the peptone or peptone-like body is decomposed.

Abrus-poison, both globulin and albumose, produce, like snake-venom, a local lesion, viz., inflammation and œdema, with ecchymosis ; but the activity of venom in producing this result is enormously greater than that of abrus. It is not the globulin alone of abrus that produces the local lesion, but also the albumose. As in many cases of such poisoning, also, the blood after death is in a fluid or semi-fluid state.

The effect of heat on abrus-poison is more marked and definite than on snake-venom. The physiological activity of the globulin is, *e.g.*, completely destroyed at about its coagulation temperature, 80°C., while the activity of the albumose is not destroyed until the solution is raised to 85° C.

Nature of Abrus-poison.—To explain the action and nature of abrus-poison, two theories may be stated :—

1. That the poison is of ferment-nature attached to the proteids.
2. That the proteids develop by contact with living tissue a body or bodies which are poisonous.

The first idea is only supported by the fact that the activity of both poisonous proteids is destroyed at about the temperature at which digestion ferments are destroyed. At the same time, there is no evidence to show that such a temperature does not so alter the constitution of the molecule of the proteids that they do not produce by contact with living tissue toxic principles. Since there is no accurate knowledge of the constitution of the proteid molecule, the question as to why one proteid should be poisonous and another harmless must remain unsettled. Although this is so, the results obtained in the experiments on the abrus-poison are definite, and may be thus summarised :—

1. The poisonous activity of the seeds of *Abrus precatorius*, the jequirity, resides in the two proteids present in the seeds—a paraglobulin and an albumose.
2. Both of these proteids have practically the same action. They produce severe conjunctivitis when applied to the eye ; and when subcutaneously injected they cause local inflammation, œdema, and ecchymosis, and gastro-intestinal irritation, with extrusion of fæces

and blood; the general symptoms being, first, a great fall of the body temperature, and a condition of stupor, ending in death.

3. The activity of both these proteids is destroyed by moist heat. In solution the activity of the globulin is destroyed at between 75° and 80° C., and that of the albumose between 80° and 85° C.

4. That abrus-poison resembles snake-venom in chemical composition, in the local lesions produced, in producing a fall of body temperature, in causing semi-fluidity or fluidity of the blood after death, and, to some extent, in the effect of moist heat on it. Abrus-poison is, however, much less active than snake-venom.

The following table shows a comparison between the activity of the venom of various snakes and of Abrus:—

<i>Vipera berus</i> (common adder) ..	Fatal dose in man 0·0021 gram per kilo. of body weight (Fontana).*
<i>Hoplocephalus curtus</i> (Austrian tiger snake)†	Fatal dose in dog, 0·00485 gram per kilo. of body weight; $\frac{1}{2}$ a grain in medium size dog (15 lbs.).
Cobra	Fatal dose in dog, 0·000079 gram per kilo. of body weight; $\frac{1}{10}$ grain in dog weighing 18 lbs. (Vincent Richards).‡
Abrus-poison :	
Globulin	Fatal dose, 0·01 gram per kilo. of body weight.
Albumose	Fatal dose, 0·06 gram per kilo. of body weight.
Peptic albumoses	Fatal dose in dog, any dose over 0·3 gram per kilo. of body weight (Pollitzer).§

IV. "On the Early Development of *Lepidosteus osseus*.—Preliminary Notice." By J. BEARD, Ph.D., B.Sc., Zoologist to the Scottish Fishery Board, Edinburgh. Communicated by Professor T. H. HUXLEY, F.R.S. Received April 20, 1889.

In the spring of 1888 I journeyed to North America for the purpose of collecting material for a study of Ganoid development.

I sought and found even more material than I wanted in the now well-known habitat of *Lepidosteus*, Black Lake, N.Y. No better hunting-ground could be wished for by the morphologist in search of Ganoid material. The lake contains *Amia*, multitudes of *Lepidosteus*, and (it is said) sturgeons. One need not be at much trouble in seeking sturgeons, for the River St. Lawrence, which flows within 12 miles of Black Lake, will vie with any Russian river. I made the

* Quoted in Marx, 'Gift-Lehre,' vol. 2, p. 74.

† "Report of Special Commission on Snake-poisoning." 'Australian Med. Journal,' 1876, No. 21, p. 104.

‡ 'Landmarks of Snake-poison Literature.'

§ 'Journal of Physiology,' 1886.

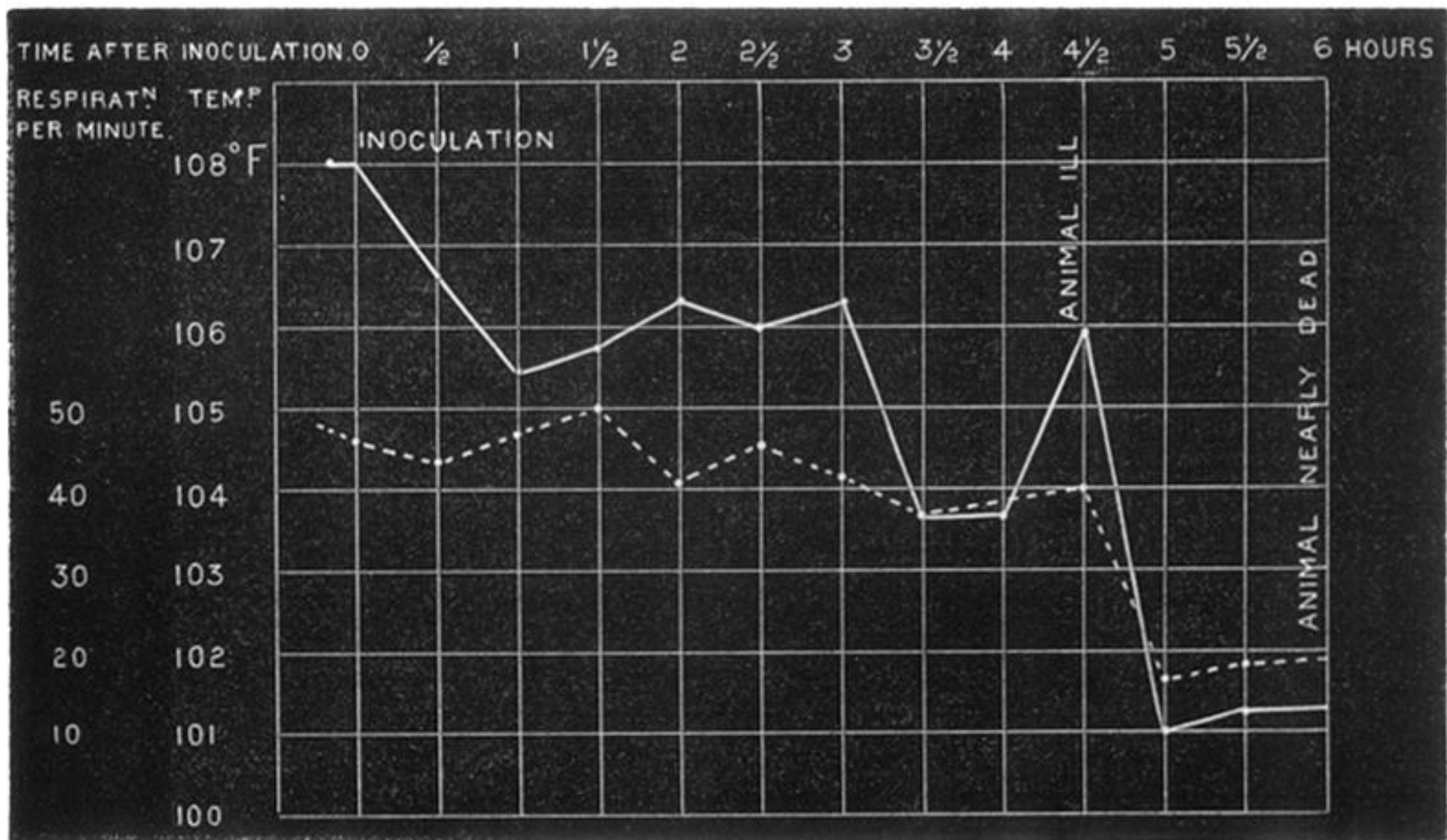


Figure showing effect of Abrus-albumose on temperature and respirations of the pigeon. Temperature taken in rectum every half-hour. Dotted line, respiration; thick line, temperature.