Study XXII : the effect of theobromin sodium salicylate in acute experimental nephritis, as measured by the excretion of phenolsulphonephthalein / Henry A. Christian, M.D., Boston.

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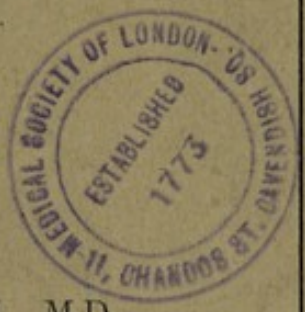

HENRY A. CHRISTIAN, M.D.
BOSTON

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Study XXII: The Effect of Theobromin Sodium Salicylate in Acute Experimental Nephritis, as Measured by the Excretion of Phenolsulphonephthalein

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STUDY XXII: THE EFFECT OF THEOBROMIN SODIUM SALICYLATE IN ACUTE EXPERIMENTAL NEPHRITIS, AS MEASURED BY THE EXCRETION OF PHENOLSULPHONEPHTHALEIN *

HENRY A. CHRISTIAN, M.D.
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The therapeutic efficacy of diuretic drugs on patients with acute nephritis has been discussed by most writers in their description of methods of management of acute nephritis. The views expressed have been based largely on clinical observation, and there has been relatively little experimental evidence[1] accumulated in support of the claims made for the usefulness, within certain limitations, of diuretic drugs in acute nephritis. Inasmuch as clinical observation of acute nephritis is much hampered by the infrequency of uncomplicated cases and by the difficulties in determining whether any two patients actually present comparable renal lesions, it has seemed desirable to have more observations under experimental conditions in which some of the factors are controllable in the hope that gradually data may be accumulated as a basis for a more efficient method of treating acute nephritis. The following experiments are reported for this reason. Though inconclusive in many respects, it is hoped that they may throw some slight light on the problem and at least be helpful to other investigators by showing what has happened to these animals under the conditions of the experiment.

The experiments here reported concern themselves with one diuretic (theobromin sodium salicylate or diuretin*) used in one form of

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* From the Laboratory of the Theory and Practice of Physic, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.

* This is one of a series of studies on experimental cardiorenal disease. Study I, Smith: Boston Med. and Surg. Jour., 1908, clviii, 696; Study II, Christian: Boston Med. and Surg. Jour., 1908, clix, 8; Study III, Christian: Jour. Am. Med. Assn., 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker: The Archives Int. Med., 1911, viii, 468-551; Study XVI, Christian and O'Hare: The Archives Int. Med., 1913, xi, 517; Studies XVII and XVIII, O'Hare: The Arhives Int. Med., 1913, xii, 49, 61; Study XIX, Christian and O'Hare: Jour. Med. Research, 1913, xxviii, 227; Study XX, Walker and Dawson: The Archives Int. Med., 1913, xii, 171; Study XXI, Fitz: The Archives Int. Med., 1914, xiii, 945.

1. See Study XXI by R. Fitz (The Archives Int. Med., 1914, xiii, 945) for a report of the immediate effects of theobromin sodium salicylate and theocin in experimental nephritis and for a review of other experimental work on the effects of diuretics.

* The preparation used throughout these experiments was made by Knoll & Co., and purchased from retail druggists under the name "diuretin."

acute nephritis (that produced by uranium nitrate) in a single laboratory animal (rabbit). Forty-nine animals were utilized and they were kept under similar conditions in metabolism cages on a diet of carrots, oats and water. The amount of each food taken by the rabbit varied with the animal's appetite, but all were measured. The urine was collected and measured in seventy-two- or twenty-four-hour periods. The uranium nitrate was given subcutaneously in doses of 3.5, 1.1, 0.5 and 0.25 mg. per kilogram of body weight, respectively, to different groups of animals. Forty-eight hours later theobromin sodium salicylate was

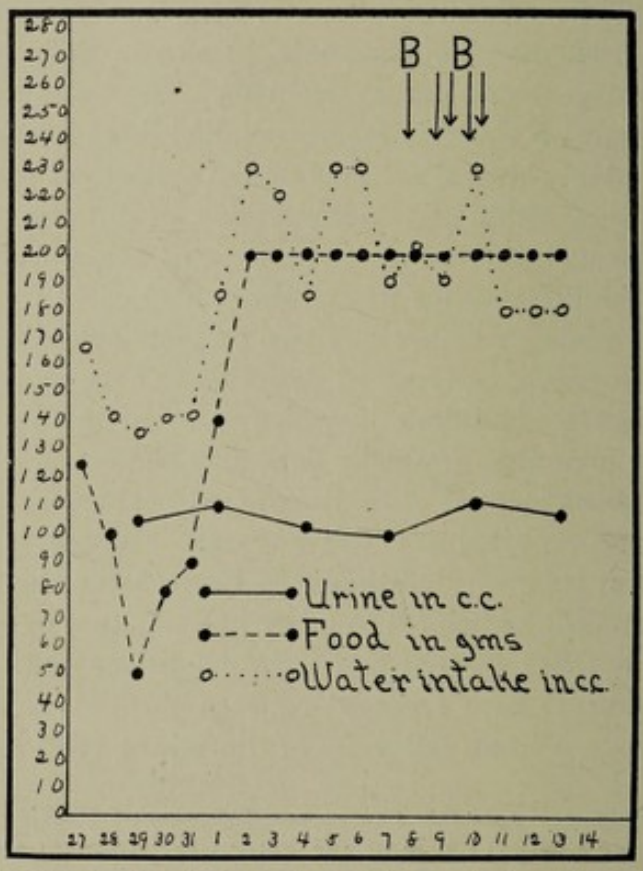


Chart 1.—Rabbit 86. *B*, theobromin sodium salicylate, 14 mg. per kilogram of body weight.*

given subcutaneously to a portion of these animals in a dose (14 mg. per kilogram of body weight) comparable to the therapeutic dose for man, and this was repeated in most cases twice daily for several days. The phenolsulphonephthalein excretion was determined at intervals; the phenolsulphonephthalein was given intramuscularly in a dose of 6 mg., and the urine for quantitation of the phenolsulphonephthalein excretion was obtained by catheterization with thorough washing out of the bladder; quantitation was carried out by the usual colorimetric method.

*Numerals along bottom of charts indicate days of the month.

The rabbits were studied in small groups at different periods between May, 1913, and April, 1914, and these groups were obtained from several dealers; consequently, in order to reduce the variants to a minimum, only animals within the several groups have been compared.

As a preliminary control, normal rabbits were given theobromin sodium salicylate in the way described. The result was practically nil, as shown by the chart[2] of Rabbit 86 (Chart 1). Other animals showed the same thing.

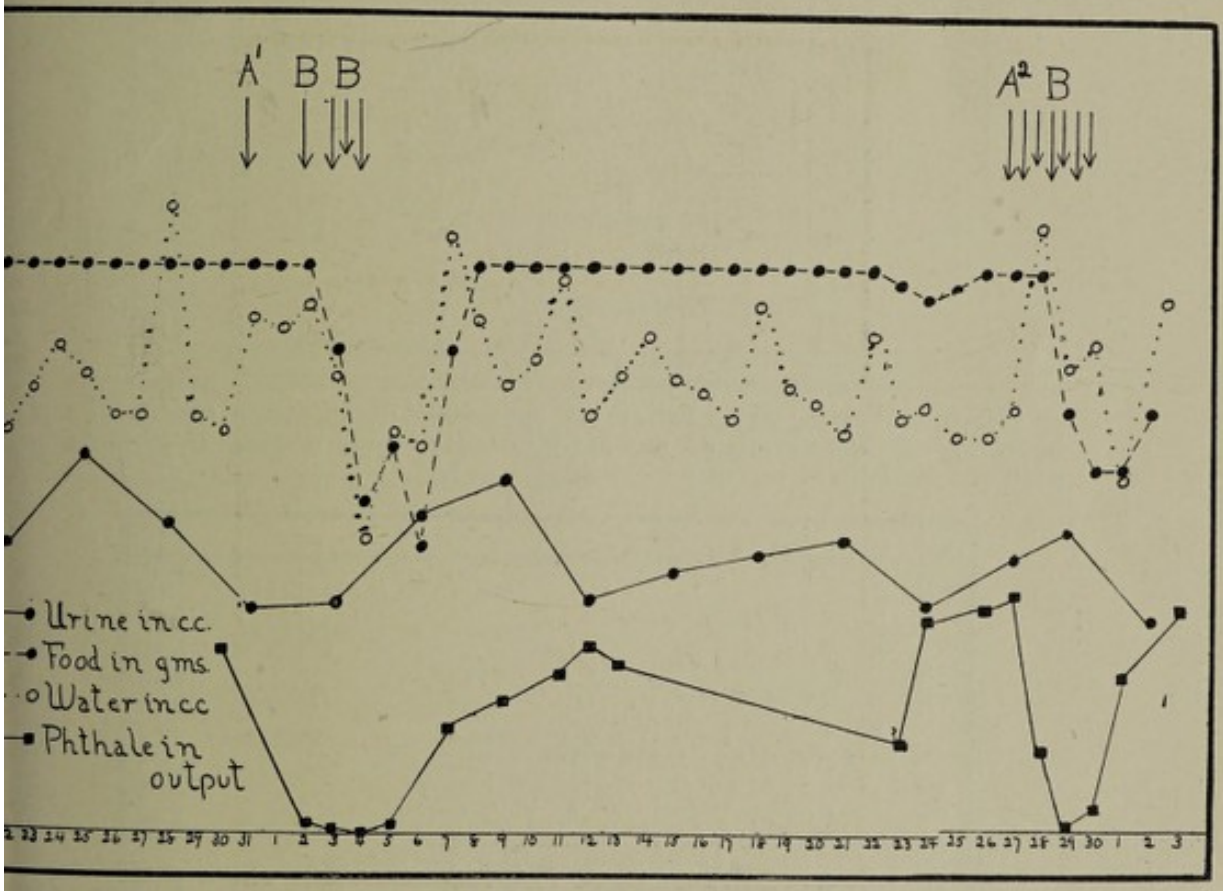


Chart 2.—Rabbit 686. Period of phenolsulphonephthaein excretion, one hour, 33 minutes. A^1, uranium nitrate, 3.5 mg. per kilogram of body weight. A^2, uranium nitrate, 1.1 mg. per kilogram. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

In the first series 3.5 mg. of uranium nitrate per kilogram of body weight was given to six animals (Rabbits 686, 687, 688, 689, 691 and 692) with results as shown in illustrative charts of a few rabbits[3] (Charts 2 and 3). This amount of uranium nitrate produces a severe

2. In all charts "water intake" includes water content of carrots calculated as 88 per cent. of total weight of carrots eaten.

3. Owing to expense of reproduction only a few of the charts which were made of each rabbit have been printed.

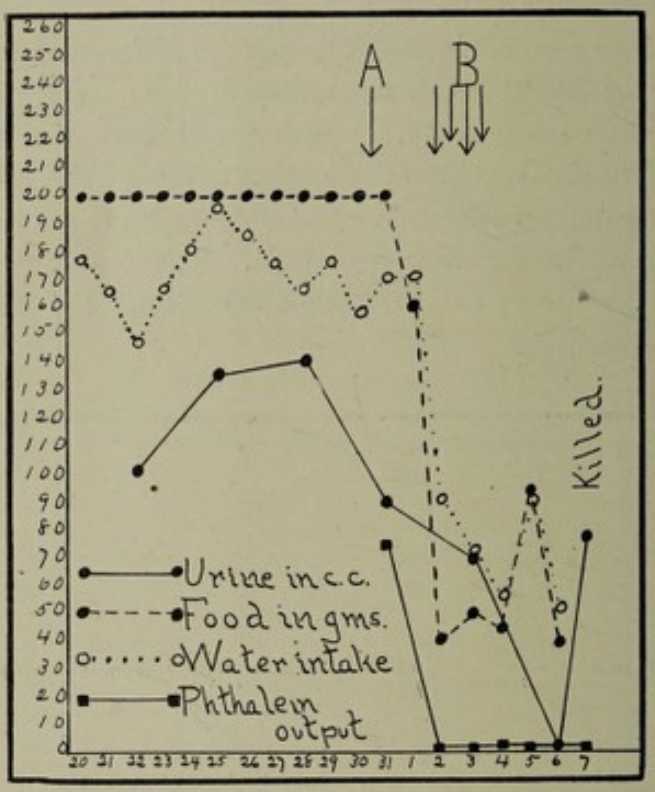


Chart 3.—Rabbit 688. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 3.5 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

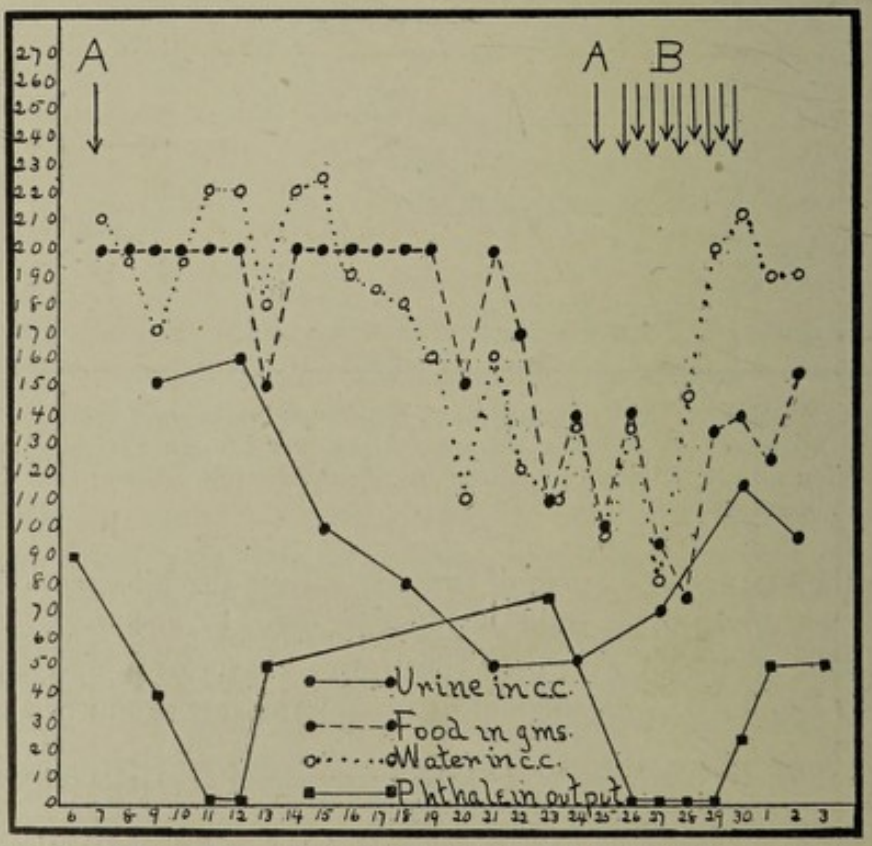


Chart 4.—Rabbit 701. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 1.1 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

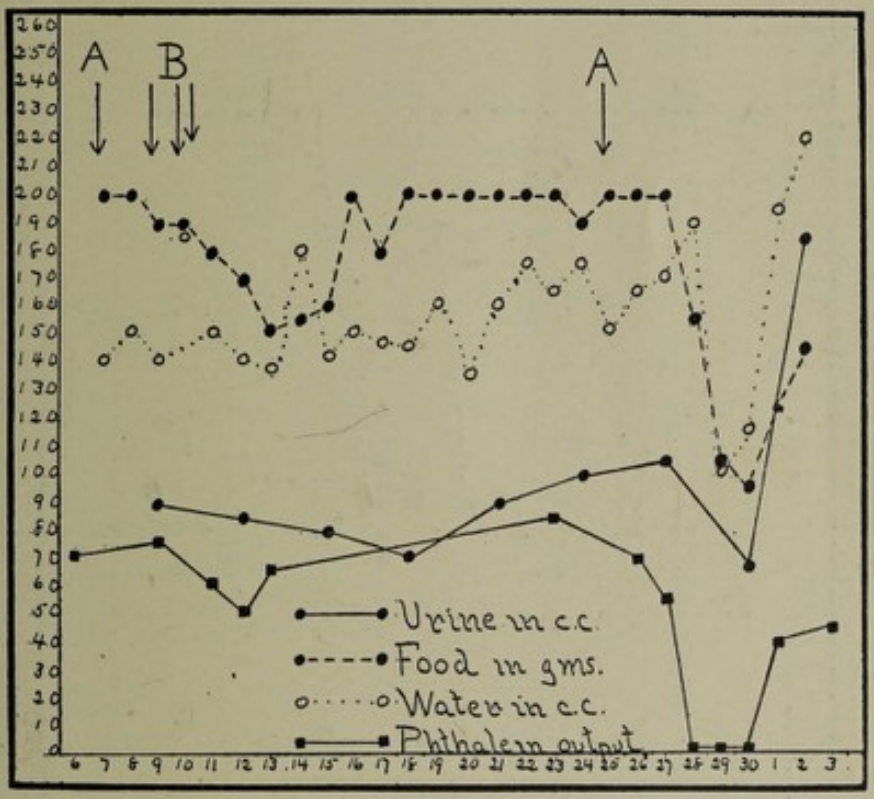


Chart 5.—Rabbit 702. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate 1.1 per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

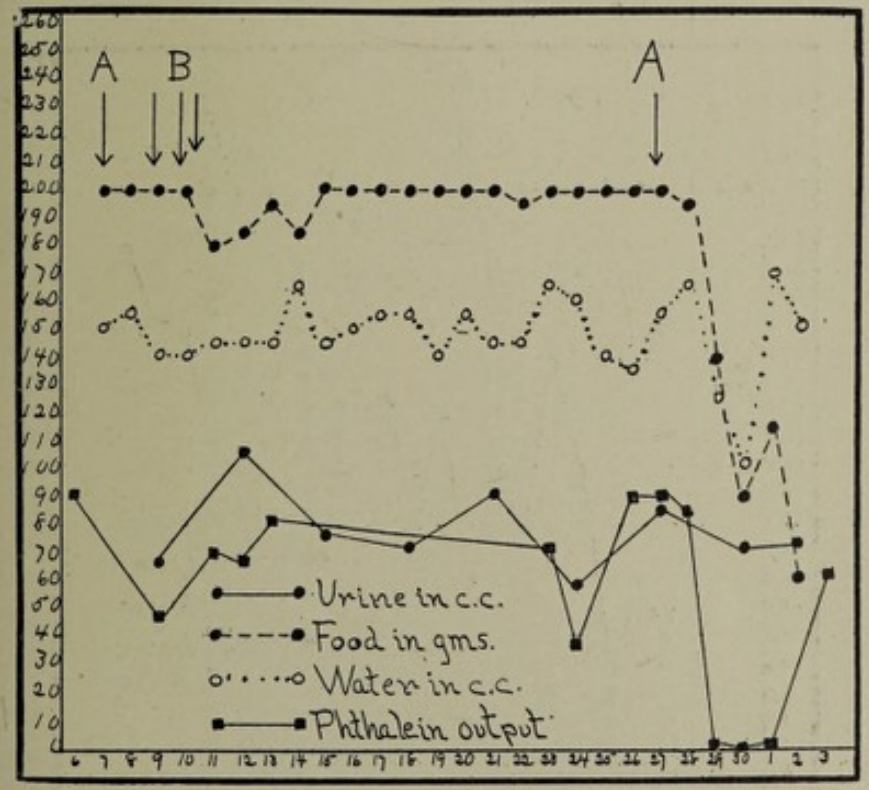


Chart 6.—Rabbit 705. Period of phenolsulphonephthalein excretion, one hour, thirty minutes. *A*, uranium nitrate, 1.1 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

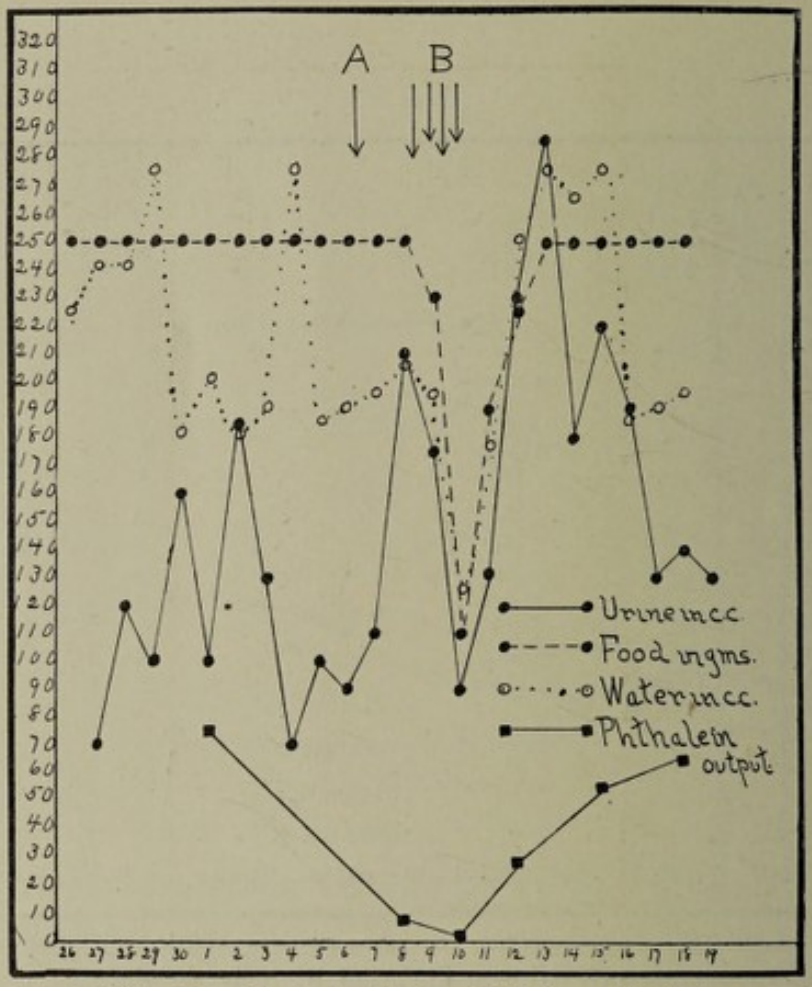


Chart 7.—Rabbit 816. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 0.5 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

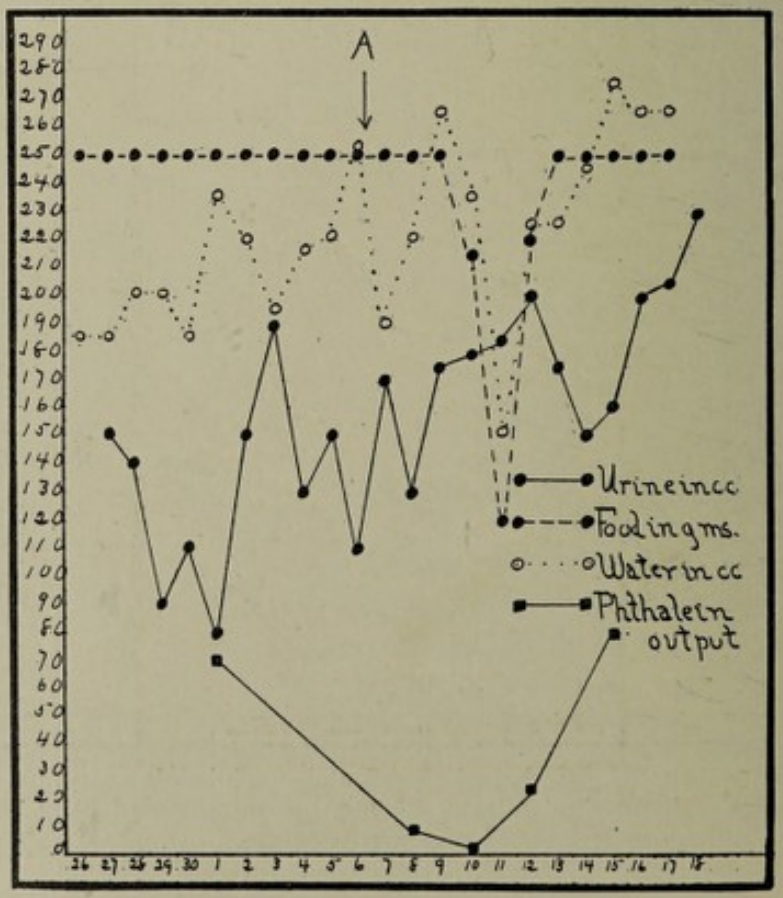


Chart 8.—Rabbit 819. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 0.5 mg. per kilogram of body weight.

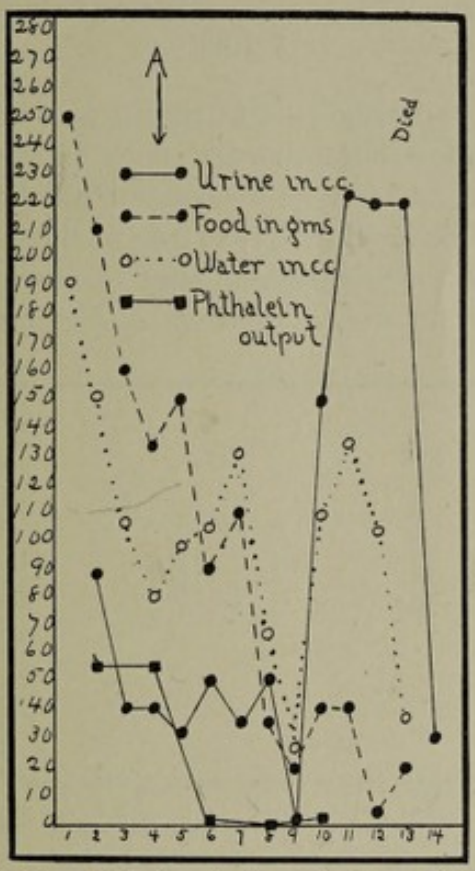


Chart 9.—Rabbit 790. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 0.5 mg. per kilogram of body weight.

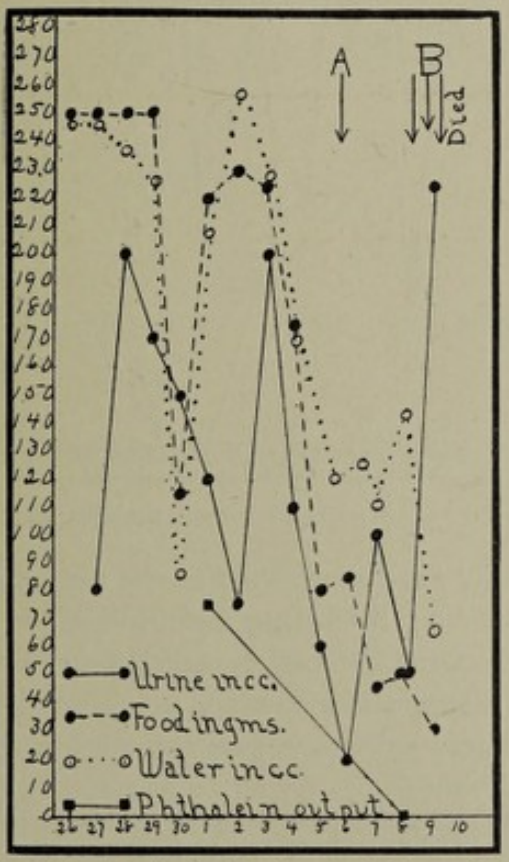


Chart 10.—Rabbit 815. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 0.5 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

usually fatal acute nephritis (see Christian and O'Hare[4] for average duration of life and lesion produced with and without theobromin sodium salicylate), and in these six rabbits all died in a short time, except one (Rabbit 686, Chart 2), to which theobromin sodium salicylate was given and which subsequently survived a smaller dose of uranium nitrate. In the five animals which died, those with and with-

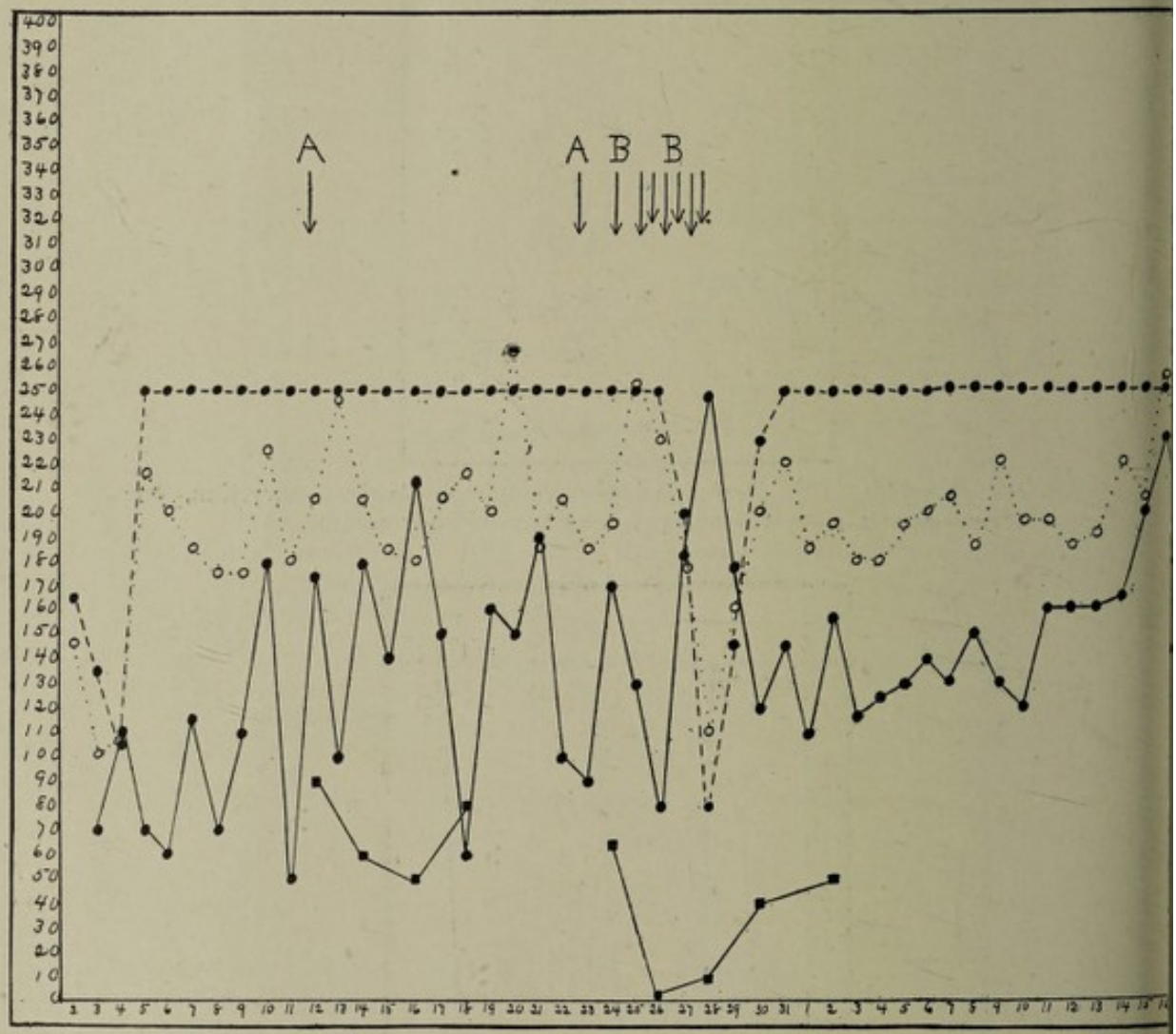
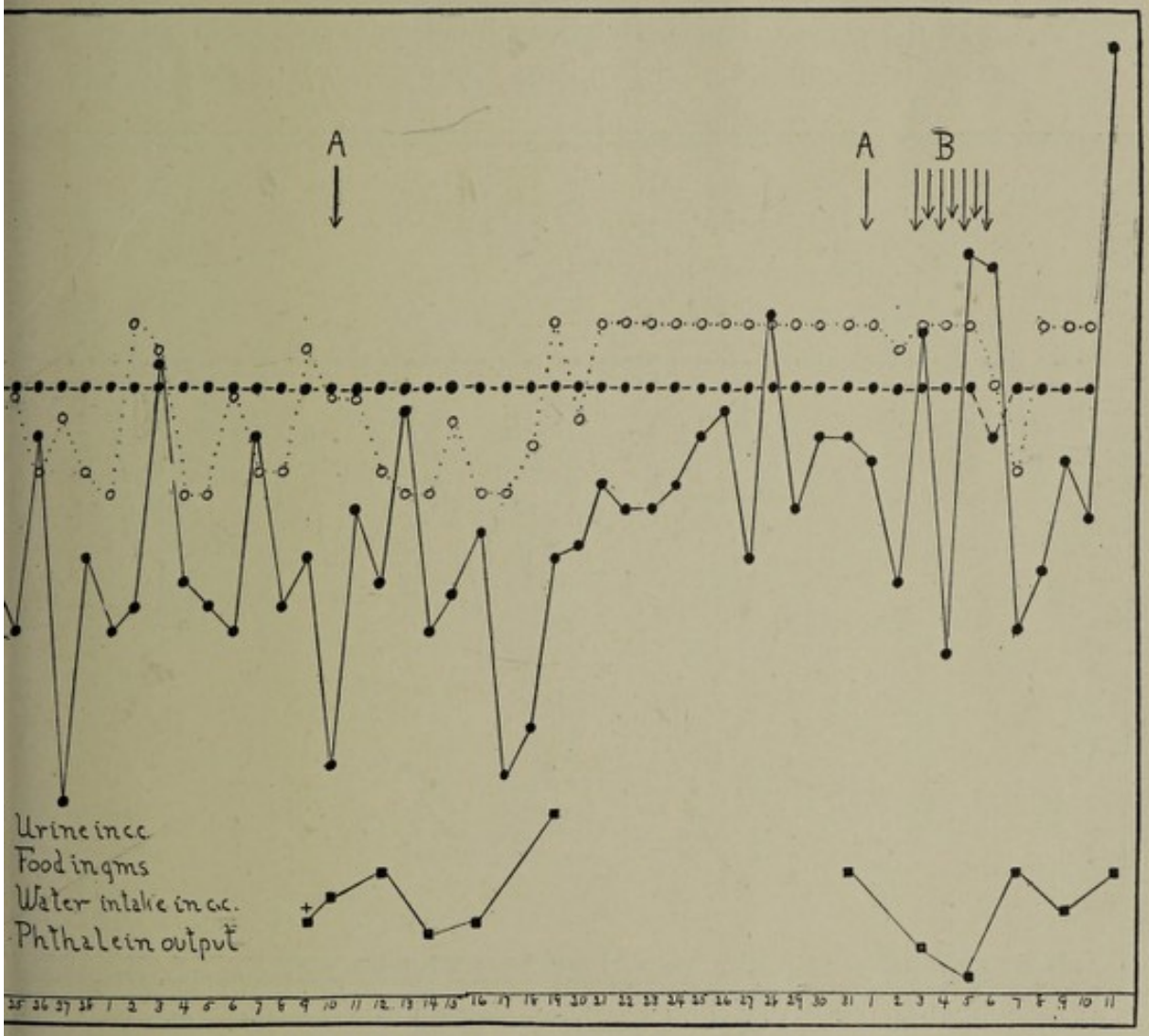


Chart 11.—Rabbit 849. Period of phenolsulphonephthalein excretion, one hour. *A*, u

out theobromin sodium salicylate showed the same curve of phenolsulphonephthalein excretion, indicating a marked disturbance of function uninfluenced by theobromin sodium salicyate, though in one rabbit (688, Chart 3) a diuresis immediately preceded death. Whether or not theobromin sodium salicylate was responsible for the survival of

4. Christian, Henry A., and O'Hare, James P.: A Study of the Therapeutic Value of a Diuretic (Theobromin Sodium Salicylate or Diuretin) in Acute Experimental Nephritis (Study XVI), The Archives Int. Med., 1913, xi, 517.

Rabbit 686 cannot be determined. It is known that rabbits vary in their susceptibility to uranium nitrate, and this may represent an unusual degree of natural resistance. This was observed in several rabbits of a previous series (Christian and O'Hare[4]), although in most of the rabbits in that study with severe nephritis theobromin sodium salicylate shortened life.



25 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

In the second (Rabbits 701, 702, 703, 704 and 705), third (Rabbits 707-708), fourth (Rabbits 709-716) and fifth (Rabbits 744-752) series of animals 1.1 mg. of uranium nitrate per kilogram of body weight was given, with results as shown in illustrative charts of some of the rabbits (Charts 4, 5 and 6). In two series the urine was collected at seventy-two-hour intervals, and in two at twenty-four-hour intervals. Of the second series, Rabbits 701, 702 and 705 received uranium nitrate twice nine days apart, once followed by theobromin sodium salicylate. These

rabbits (701, 702 and 705, Charts 4, 5 and 6) suggest a slight beneficial effect of theobromin sodium salicylate on renal function as measured by phenolsulphonephthalein excretion, but this effect is apparent rather than real when allowance is made for a probably more intense action of the second dose of uranium in an animal that a short time previously has had an acute renal lesion produced by the same dose of uranium nitrate.

If the curve of phenolsulphonephthalein excretion in all of the twenty-four animals of Series 2 to 5 is studied, it is seen that in a

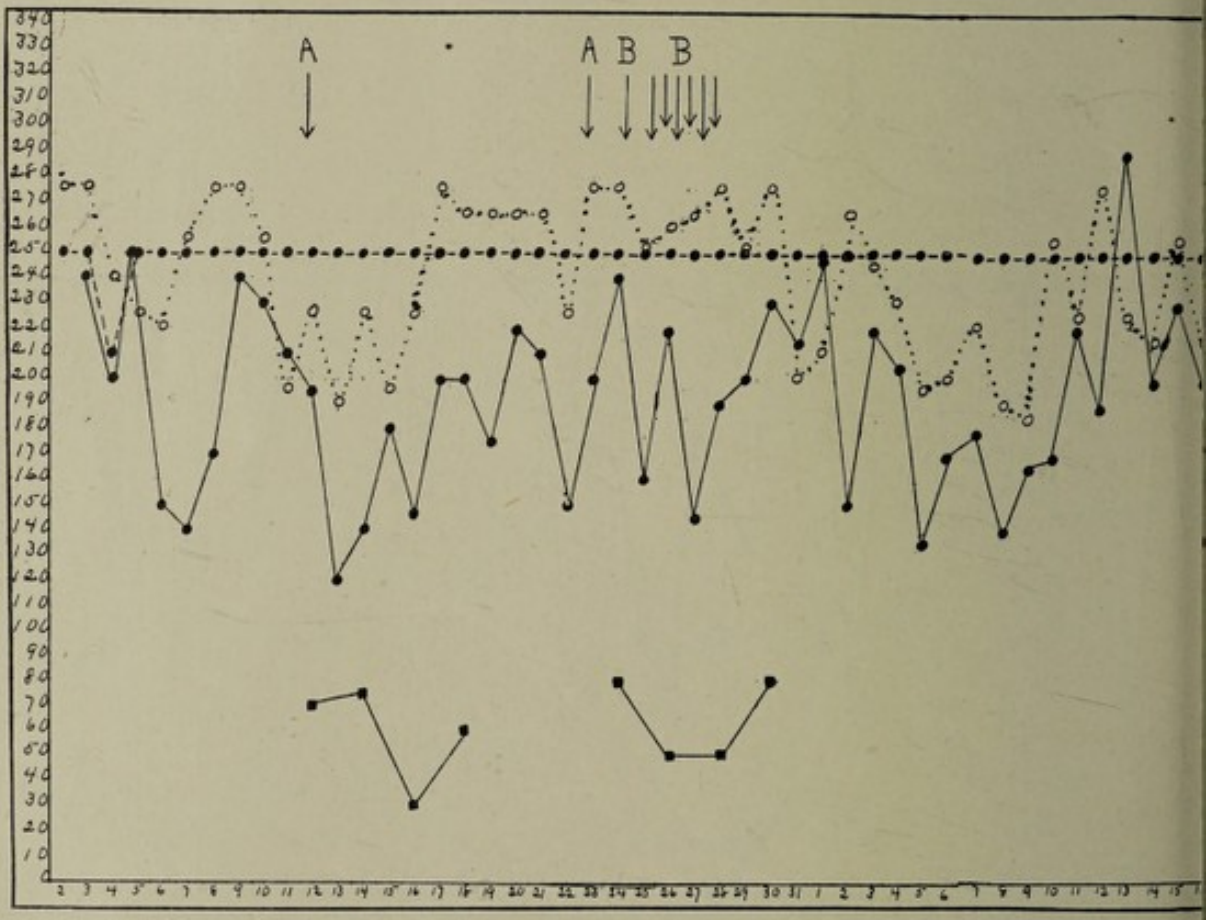
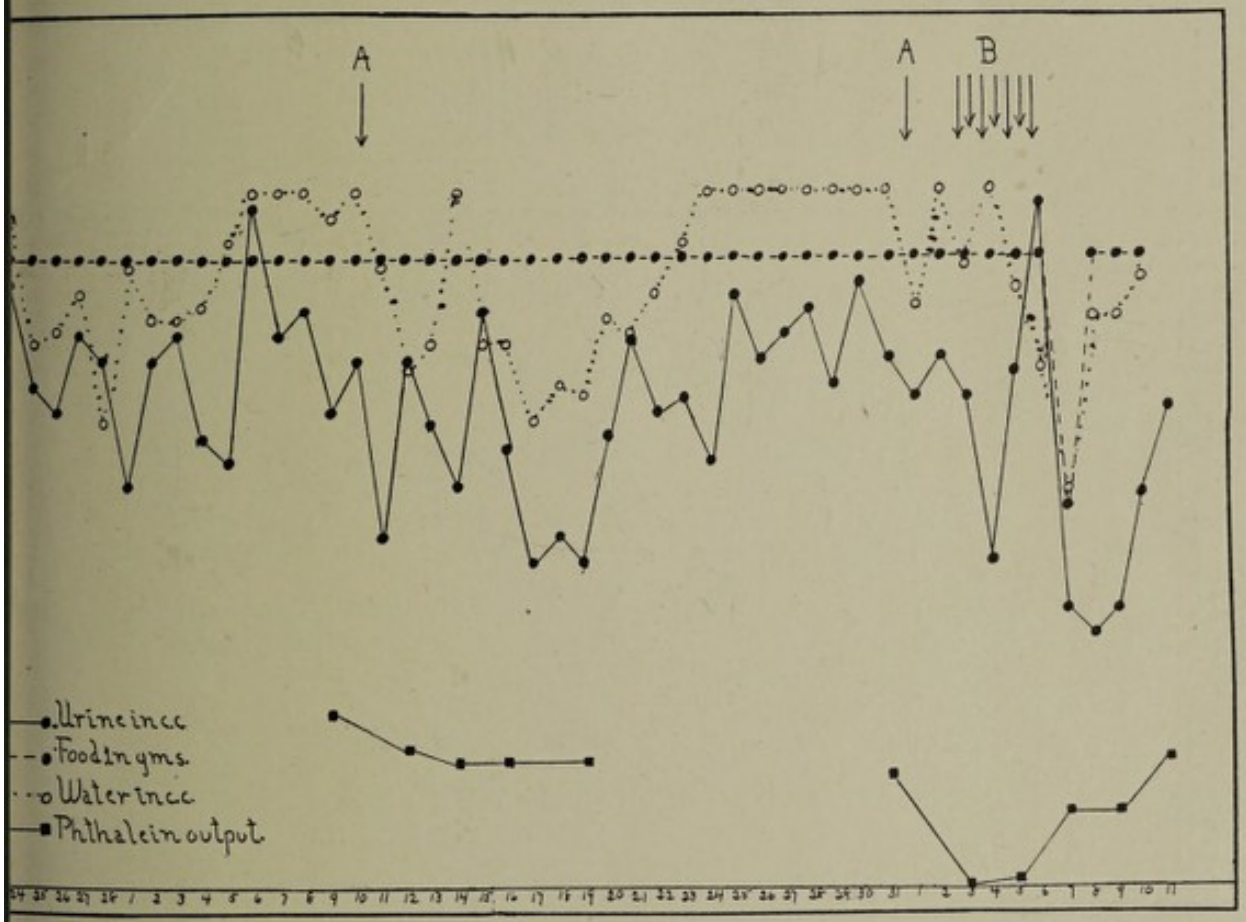


Chart 12.—Rabbit 850. Period of phenolsulp honephthalein excretion, one hour. *A*,

number theobromin sodium salicylate appears to have a slight beneficial action as expressed either by a slowed fall or a more prompt return to the normal phenolsulphonephthalein output. This is offset, however, by the fact that of the six rabbits that died, four had been given theobromin sodium salicylate. The amount of uranium given these rabbits, 1.1 mg. per kilogram of body weight, caused a severe nephritis in most, as shown by the 25 per cent. mortality, the marked loss of appetite, the decreased urine output, and the fall in phenolsulphonephthalein output to mere traces. In severe acute nephritis as shown by these series, as

well as by Series 1, it does not appear that theobromin sodium salicylate exerts any evident beneficial action on renal function as measured by phenolsulphonephthalein excretion; occasionally it seems to do good, but on the other hand, occasionally it seems to do harm. Consequently, it may be said that its use in rabbits with so severe an acute nephritis is a questionable therapeutic measure.

Examination of the charts shows that diuresis, though common, is not constant under the conditions of these experiments, and that it may occur a few days after the uranium is given, whether or not a diuretic



, 0.25 mg. per kilogram of body weight. *B*, theobromin salicylate, 14 mg. per kilogram.

is used. What is quite striking is that urine output and renal function as measured by phenolsulphonephthalein excretion do not necessarily run parallel, and sometimes animals with marked diuresis die.* In these animals phenolsulphonephthalein output is distinctly a better prognostic sign than urine output.

In Series 6 (Rabbits 789, 790, 791) and 7 (Rabbits 815-819) still smaller doses of uranium nitrate were used, 0.5 mg. per kilogram of body weight (for results see Charts 7, 8, 9 and 10 of illustrative ani-

* None of the charts selected for printing from these series show this but it is shown in Charts 9 and 10 of the next series.

mals). Two rabbits, 790 and 815 (Charts 9 and 10), one with and one without theobromin sodium salicylate, show a marked diuresis preceding death. In these series the charts show a slightly more evident beneficial effect from theobromin sodium salicylate, though this is really too slight to be considered of much importance and might be easily a matter of coincidence rather than cause and effect. Since the lesion in most of these animals is a severe one, for three out of nine died, one having received theobromin sodium salicylate, two not, it seemed desirable to use still smaller doses of uranium nitrate.

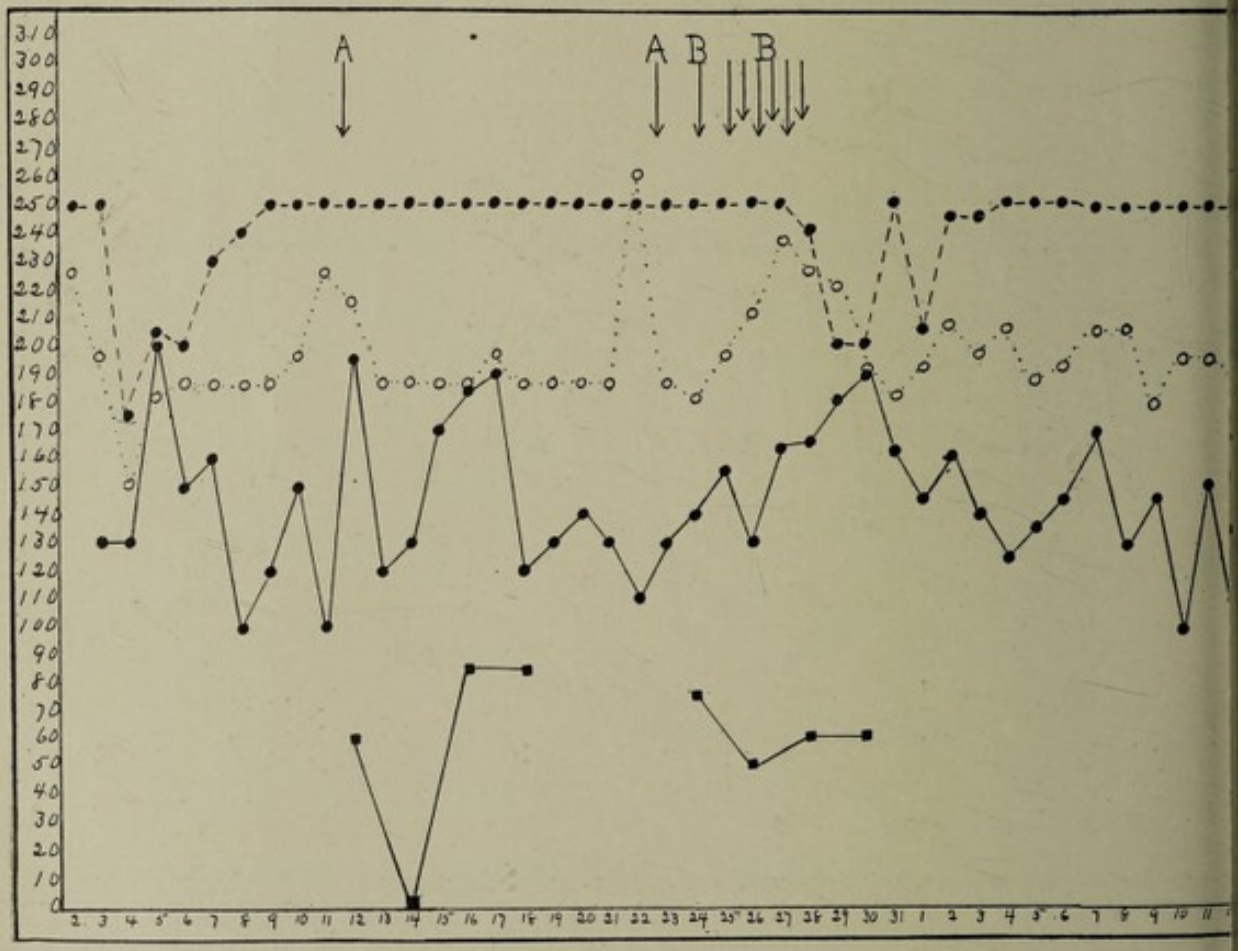
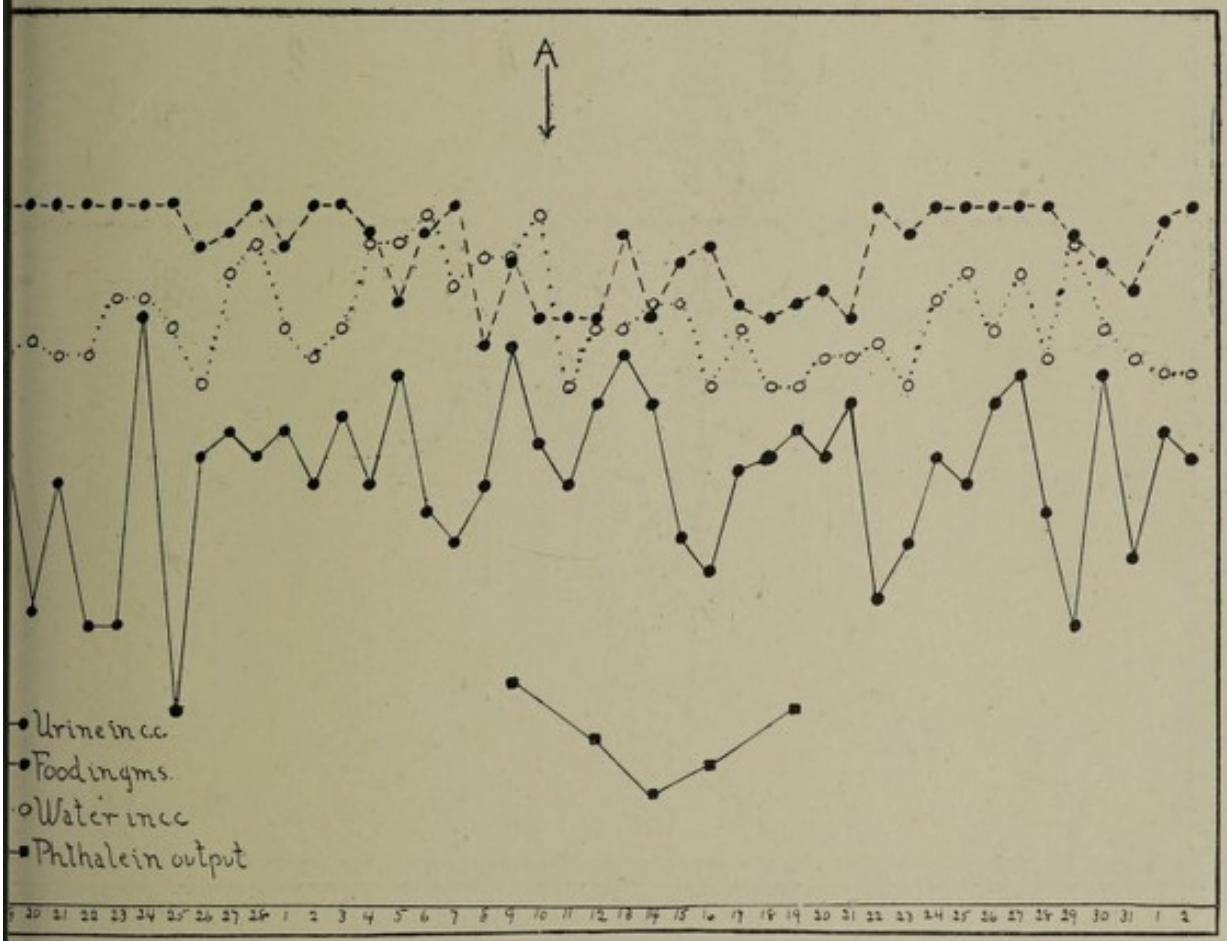


Chart 13.—Rabbit 851. Period of phenolsulphonephthalein excretion, one hour. *A*,

In the last series (Series 8, Rabbits 849-854) 0.25 mg. of uranium nitrate per kilogram of body weight was used. Inasmuch as it seemed likely that this dose would not produce any profound change in the kidney not easily recoverable from, a slightly different method was followed in that uranium was repeated after a time interval thought to be long enough for recovery. With the first dose of uranium no rabbit received theobromin sodium salicylate, and this dose was used only as a test of the rabbit's susceptibility to uranium. One rabbit, 853, died even after this small dose of uranium. This animal showed a precritical

diuresis. Rabbits 849, 850, 851, 852 and 854 (Charts 11, 12, 13, 14 and 15) showed a very slight disturbance of renal function as evidenced by decreased output of phenolsulphonephthalein. That the disturbance was slight is shown by the prompt return to normal of the phenolsulphonephthalein excretion and the failure of the rabbits to have any decrease in appetite. One rabbit (852, Chart 14) showed no decrease in phenolsulphonephthalein, but a slight loss in appetite. After ten days, uranium was repeated to the five remaining rabbits, and to three of these theobromin sodium salicylate was subsequently given. In these, in general theobromin sodium salicylate appeared to decrease slightly



0.25 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

functional disturbance as evidenced by phenolsulphonephthalein excretion, but not to have much effect as indicated by the animals' appetite. All five animals showed moderately increased diuresis subsequent to the uranium, but this diuresis was not definitely influenced by theobromin sodium salicylate.

Forty-six days following the second dose of uranium, the same animals received a third dose of uranium. To the two not receiving theobromin sodium salicylate previously this drug was given. In

Rabbits 849 (Chart 11) and 850 (Chart 12) without theobromin sodium salicylate, phenolsulphonephthalein excretion was slightly less depressed after uranium than had previously been the case when theobromin sodium salicylate had followed the uranium. In Rabbit 851 (Chart 13) the reverse was true. In Rabbit 852 (Chart 14) with theobromin sodium salicylate the curve of phenolsulphonephthalein excretion showed a delayed fall and a slightly more abrupt rise than previously

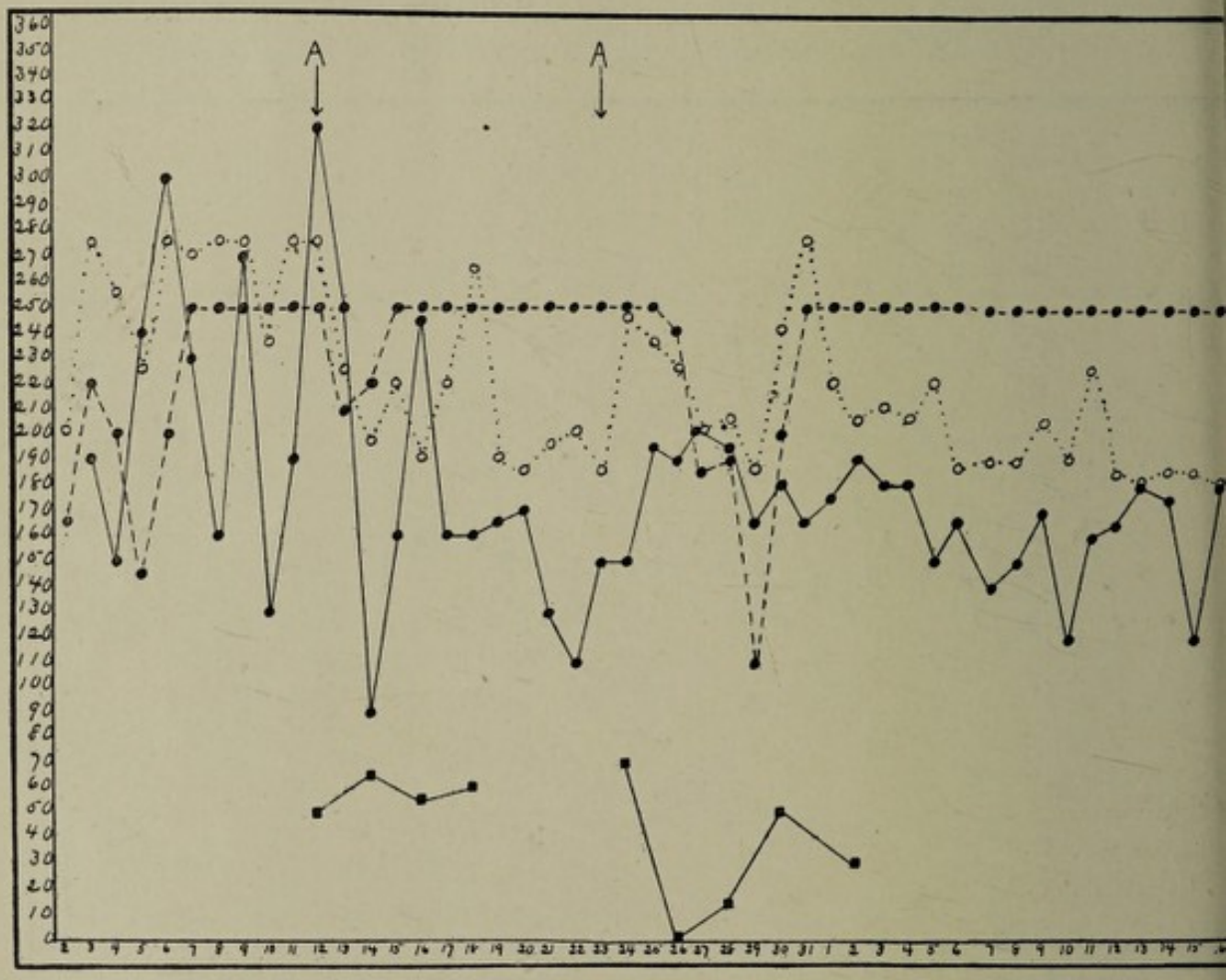


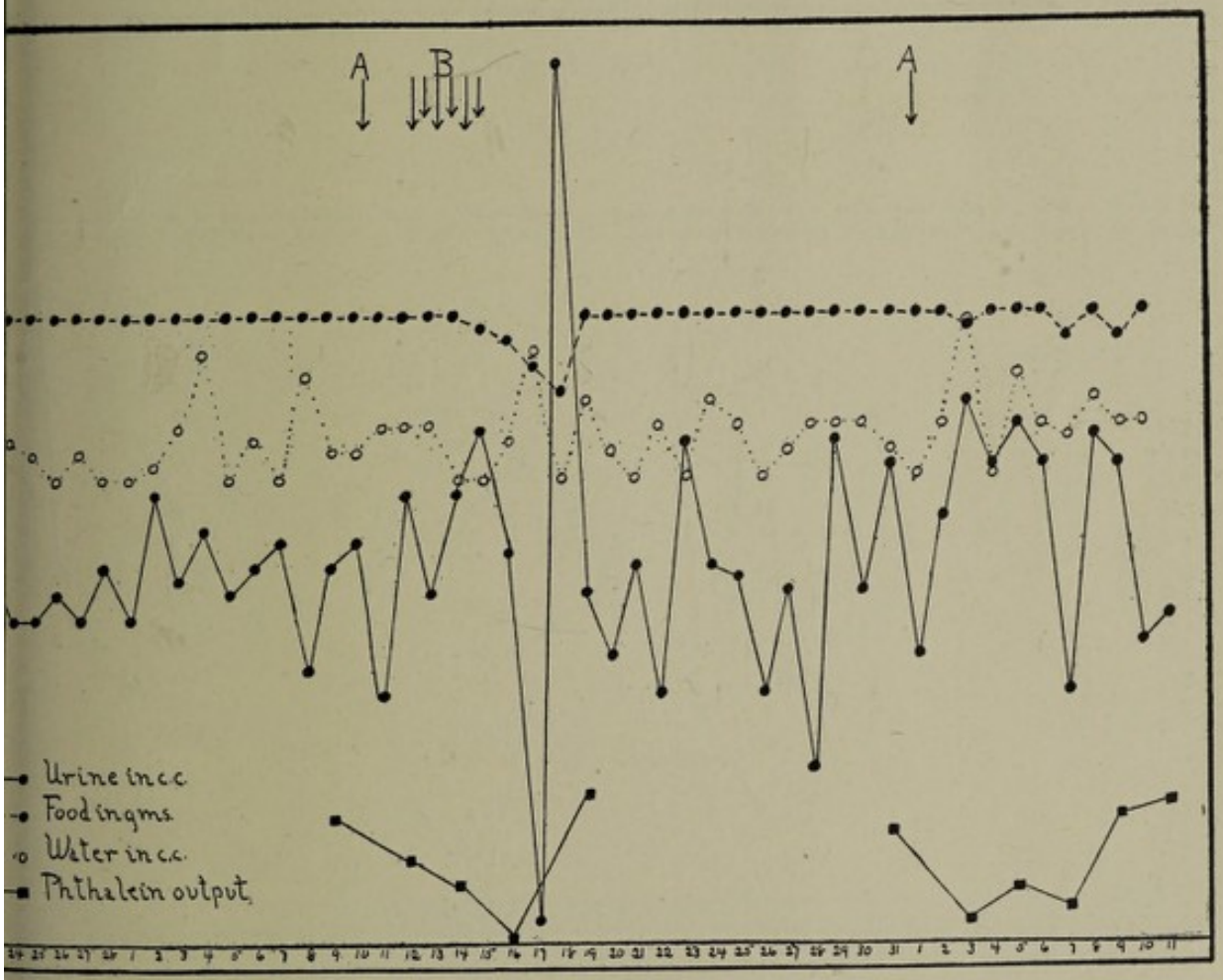
Chart 14.—Rabbit 852. Period of phenolsulphonephthalein excretion, one hour. *A*,

without theobromin sodium salicylate, while in Rabbit 854 (Chart 15) with theobromin sodium salicylate the phenolsulphonephthalein curve showed much less of a fall than had been the case in this rabbit without theobromin sodium salicylate.

If we compare the phenolsulphonephthalein excretion with and without theobromin sodium salicylate in these rabbits after the second and third dose of uranium, the curves taken all together show a slightly better excretion with theobromin sodium salicylate than without it. The difference, however, is slight, for it appeared in favor of theo-

bromin sodium salicylate in but three out of five experiments, and in these the differences were quantitatively slight.

Finally, in three rabbits, 849, 850 and 852 (Charts 11, 12 and 14) a fourth injection of uranium was given twelve days following the third, and after this dose theobromin sodium salicylate was given to two animals. Here the curves were distinctly though slightly against this diuretic improving renal function.



; 0.25 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

If the last group with very slight degrees of renal disturbance is viewed as a whole and if allowance is made for some probable variation in effect from so small a dose of uranium, to say nothing of variations in results obtained at times without explainable cause in all of the observations here reported, the conclusion would seem justifiable that even in very slight degrees of acute experimental (uranium) nephritis in the rabbit there is little evidence of any beneficial action from theobromin sodium salicylate on renal function as measured by phenolsulphonephthalein excretion. It is equally true that there is little evi-

dence of any harmful action. Rather are the results suggestive of no constant action.

SUMMARY

So far as renal function may be judged from phenolsulphonephthalein excretion in acute experimental nephritis produced by uranium in the rabbit, there is no evidence that theobromin sodium salicylate exerts any constant beneficial action. In the severer forms

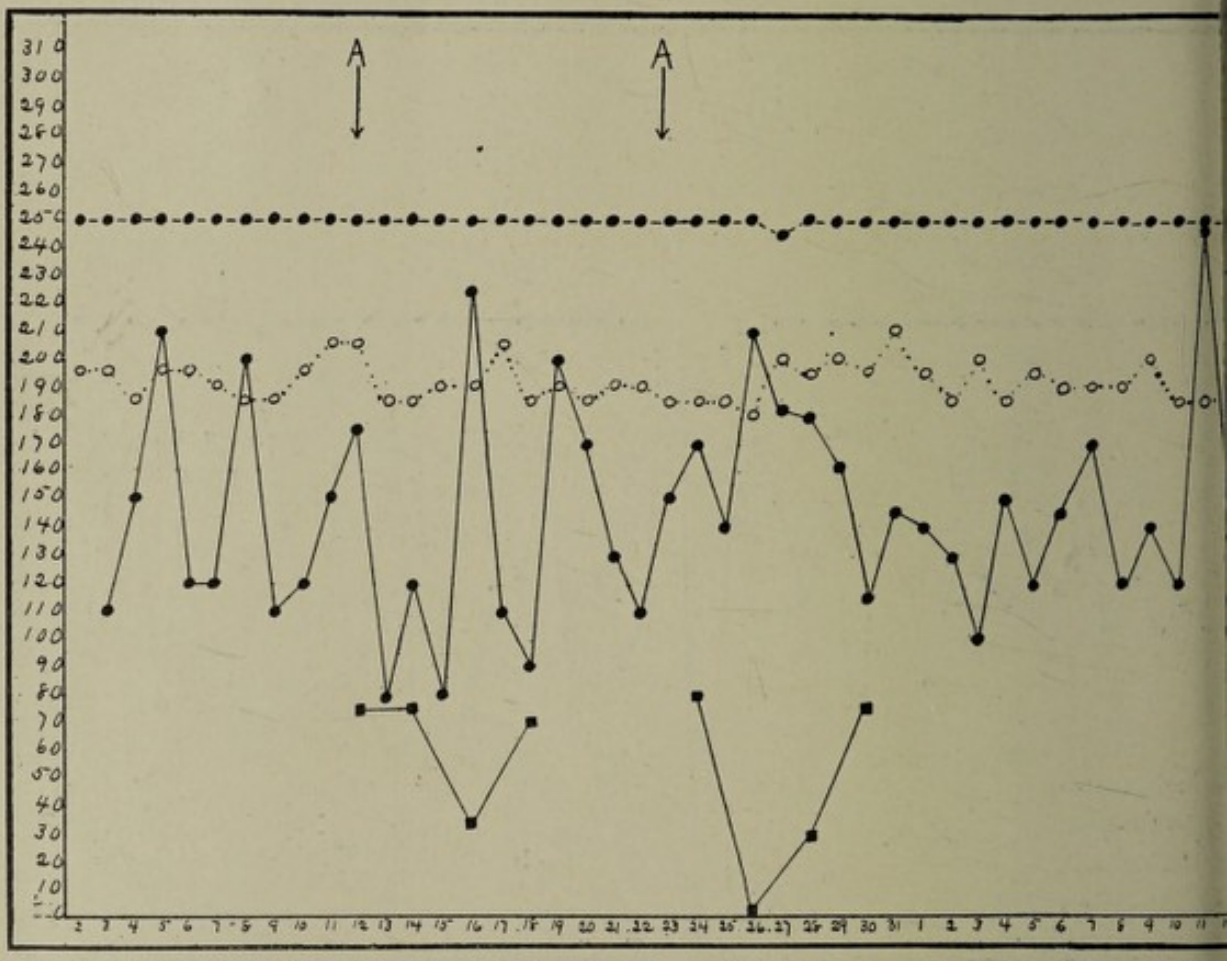
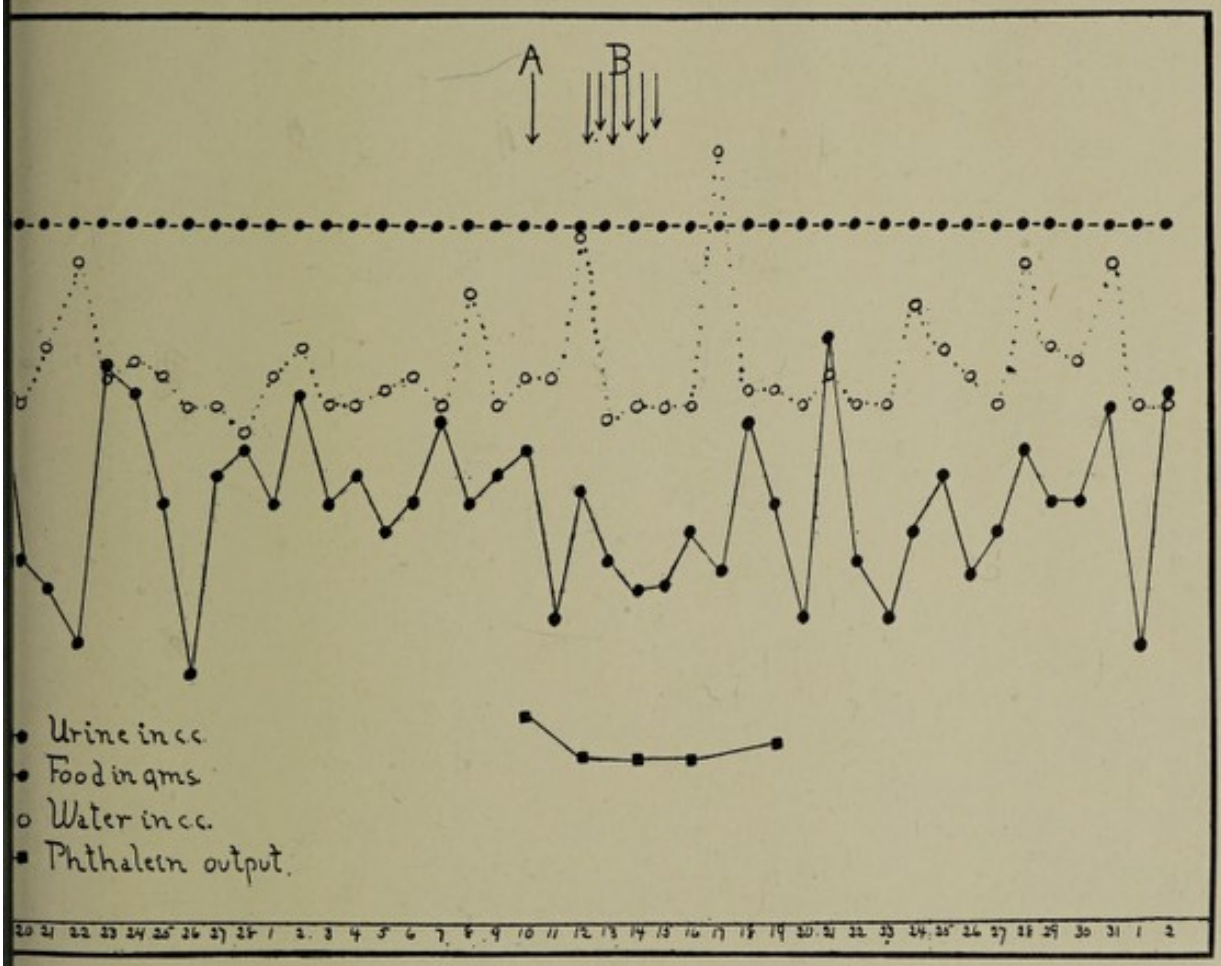


Chart 15.—Rabbit 854. Period of phenolsulphonephthalein excretion, one hour. *A*, t

there is almost no evidence of beneficial action; in the very mild forms there is occasionally evidence of beneficial action, but this is too inconstant to justify the conclusion that theobromin sodium salicylate really improves renal function even in the very mild forms of acute nephritis. As other experimental evidence (Christian and O'Hare[4]) has shown the injurious action of theobromin sodium salicylate in shortening the life of rabbits with severe acute experimental (uranium) nephritis, it would seem fair to conclude that there is considerable evidence against

the use of theobromin sodium salicylate in severe experimental nephritis, and very little in its favor in milder forms in which the conditions of experimentation involve observation over several days. It hardly seems necessary to add that it should be clearly kept in mind that the evidence adduced above concerns itself only with the rabbit, and the rabbit with acute nephritis of a single type, that produced by uranium, and that only one measure of renal function (phenolsulphonephthalein excre-



25 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

tion) is being used. The results obtained in the rabbit are in accord with the common clinical teaching that diuretics are not indicated in acute nephritis in man; but they are no proof of the correctness of this teaching, for results obtained in rabbits cannot be directly read in terms of man. However, they would seem to lend some further support to the view based on clinical observation that diuretics are not indicated in acute nephritis.

Peter Bent Brigham Hospital.