the 7-week period in which observations were made. They were fed and given the required dose of insulin daily at 9:00 a.m. and 5:00 p.m. The urine was collected under toluene and was measured and analyzed for glucose daily. Control analyses were performed for 7-day periods, alternating with 14-day periods in which 5 cc or 10 cc (250 or 500 mg) of colloidal sulphur was given daily by stomach tube.

Results. The average daily glucose outputs are shown in Table I.

TABLE I.

Dog No.	1st control period (7 days)	1st sulphur period (250 mg and 14 days)	2nd control period (7 days)	2nd sulphur period (500 mg and 14 days)	3rd control period (7 days)
I	2.76	0.87	1.22	2.64	1.47
II	5.44	2.76	3.82	3.27	1.63
III	10.52	8.31	12.13	10.81	5.21

It is evident from the above data that the administration of colloidal sulphur neither aggravated nor ameliorated the diabetic state of the animals; the glucose outputs were unchanged except for the variations ordinarily encountered. At no time was a sugar-free specimen obtained.

Conclusion. The administration of colloidal sulphur in daily doses of 250 or 500 mg has no effect on the diabetic state of the depancreatized dog.

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Tachyphylaxis to Renin.

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In the course of experiments on the preparation, purification, and assay of kidney extracts containing "renin", the phenomenon of tolerance or "tachyphylaxis" was noted. By this is meant the progressive decrease in response occurring as a result of the repeated intravenous injections of equal amounts of pressor material at equal intervals into an assay animal so prepared as to record blood pressure.

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This phenomenon has been noted by other observers.[1-11] In most instances they only comment on its occurrence or show protocols indicating its presence but provide no data. Bingel and Strauss,[2] however, suggest a means of avoiding the phenomenon.

Early in our investigation of the pressor activity of fresh kidney extracts many different preparations were used. Included among these were crude saline extracts, the products of isoelectric precipitation at various pH values, and of dialysis, ultrafiltration, and adsorption procedures. From the many animals used in testing these preparations, 21 were observed which were injected with equivalent amounts of active pressor material at various intervals. Fourteen of this group showed tachyphylaxis, 2 were doubtful and 5 showed none. The nature of the preparation did not seem to have any bearing on the appearance of tachyphylaxis. Because of the observations of other workers, our own experiences, and particularly because of the frequent need to make repeated injections into animals in the assay of active preparations, it was felt that the phenomenon should be investigated. In particular we desired to answer the following questions: (a) Does acetone used in the preparation of the "renin" give a product which causes tachyphylaxis? That is a question raised in a personal communication from Dr. Wakerlin. (b) Does the type of anesthesia of the assay animal affect the phenomenon? (c) Is a species difference between the source animal and the assay animal a factor? (d) Is the time interval between successive injections a significant variable?

Equal amounts of a standard "renin" preparation were injected intravenously into dogs whose carotid arteries were connected to a recording mercury manometer. The animals were anesthetized in various ways as indicated in the tables. An outline of the method of preparing the "renin" used follows: Fresh hog kidneys were frozen in carbon dioxide snow, crushed, extracted with 4 volumes of 5% sodium chloride for several hours, and filtered. The filtrate was

1 Bingel, A., and Claus, R., *Deutsch. Arch. f. klin. Med.*, 1910, **100**, 412.

2 Bingel, A., and Strauss, E., *Deutsch. Arch. f. klin. Med.*, 1909, **96**, 476.

3 Harrison, T. R., Blalock, A., Mason, M. F., and Williams, J. R., *Arch. Int. Med.*, 1937, **60**, 1058.

4 Hartwich, A., *Z. f. die Gesam. Exp. Med.*, 1930, **69**, 462.

5 Merril, A., Williams, R. W., Harrison, T. R., *Am. J. Med. Sci.*, 1938, **196**, 240.

6 Miller, J. L., and Miller, E. M., 1911, **43**, 242.

7 Pearce, R. M., *J. Exp. Med.*, 1909, **11**, 430.

8 Pickering, G. W., and Printzmetal, M., *Clin. Sci.*, 1938, **3**, 211.

9 Shaw, H. B., *Lancet*, 1906, **1**, 1295.

10 Thauer, R., *Cent. fur Inn. Med.*, 1934, **54**, 2.

11 Tigerstedt, R., and Bergmann, P. G., *Skand. Arch. f. Physiol.*, 1898, **8**, 223.

TABLE I.

	Rise in blood pressure, mm Hg		
	1st Inj.	2nd Inj.	3rd Inj.
Results obtained with acetone-treated and non-acetone-treated preparations. Injections at 30- to 60-minute intervals. Morphine sulphate and ether anesthesia.			
Acetone-treated preparations (10 dogs)	33	19	11
Non-acetone-treated preparations (5 dogs)	25	10	9

TABLE II.

Results obtained with various types of anesthesia. Injections at 30- to 60-minute intervals.			
Morphine sulphate and ether (15 dogs)	30	16	10
Nembutal (7 dogs)	27	16	12
Sodium barbital (2 dogs)	41	13	5
Urethane (1 dog)	45	20	4
No anesthesia—animals with pithed brain and cord (3 dogs)	46	13	7

TABLE III.

Results obtained with extracts from different species injected into assay animals of the same and of different species. Injections at intervals of 30 to 60 minutes. Morphine sulphate and ether anesthesia.			
Dog preparations into dogs (4 dogs)	22	8	6
Hog " " " (12 ")	32	20	11

TABLE IV.

Results obtained with variation in the time interval between successive injections. Anesthetics indicated.			
Less than 1 hr intervals (11 dogs) (6*, 5†)	32	19	15
1 hr intervals (11 dogs) (9,* 2†)	26	12	10
2 " " (10 ") (4,* 6†)	30	19	21
3 " " (10 ") (5,* 5†)	27	30	34
Individual results obtained in dogs injected at 3 hr int.			
Dog No. Anesthesia			
1 Nembutal	23	22	—
2 "	66	39	60
3 "	20	31	36
4 "	28	26	30
5 "	43	64	55
6 Morphine sulphate and ether	15	18	31
7 " " " "	28	40	42
8 " " " "	16	7	14
9 " " " "	16	41	16
10 " " " "	17	7	23

* Morphine sulphate and ether.
† Nembutal.

saturated with slight excess of sodium chloride, overlayed with toluene, incubated at 37°C for 24 hours, and filtered. The precipitate† was resuspended in 4 volumes of distilled water, dialyzed in a cellophane bag against tap water until the sodium chloride con-

† This precipitate was furnished through the courtesy of Dr. David Klein of Wilson Laboratories.

tent of the suspension was 3.5%, and filtered. The filtrate was acidified with hydrochloric acid to a pH of 3.5 (glass electrode) and filtered. The filtrate was mixed thoroughly with kaolin in amounts to make a 10% suspension, and filtered. The kaolin was eluted with 1% disodium hydrogen phosphate, the pH of the mixture being adjusted to 7.5 and filtered. The filtrate was treated in the cold with 7 to 10 volumes of acetone and filtered. The precipitate was dissolved immediately in small amounts of distilled water, overlayed with toluol and kept in the refrigerator. A similar concentrate of dog kidneys was prepared except that in the original 5% saline extraction, freshly minced dog kidneys were substituted for the frozen tissue. The kaolin eluate was injected and compared with the kaolin eluate of hog kidney (Table III).

The standard "renin" preparation described is such that the injection of an amount containing 0.4 mg per kilo (30 gamma nitrogen content) produces a rise of approximately 30 mm of mercury in a dog anesthetized with morphine-ether. Approximately 300 g of fresh hog kidney yield 1 g of dried active substance.

Of the factors above noted, the only one which seems to have any significance pertaining to the appearance of tachyphylaxis is the time interval between successive injections. Study of the data in Table IV indicates that as the interval between the injections is increased, tachyphylaxis becomes less marked. In the dogs injected at 2-hour intervals, some tolerance is apparent, whereas, in the dogs injected at 3-hour intervals, a suggestion of some augmentation is evident. The importance of the time interval was recognized by Bingel and Strauss[2] who, injecting a press juice made from rabbit kidneys into a rabbit under urethane anesthesia, report that tolerance is abolished by waiting 2 hours between injections.

Conclusions. 1. A method is described which will give a reliable, relatively stable, active "renin" preparation. 2. The method of preparing the "renin", the use of acetone in its preparation, the type of anesthesia used for the assay animal, species difference between source and assay animal, do not appear to affect significantly the appearance of the phenomenon of "tachyphylaxis" or tolerance. 3. The time interval between successive injections does significantly affect the appearance of tachyphylaxis, and the effect is more pronounced the shorter the interval.[2] The effect is least noticeable when the interval is 120-180 minutes. 4. Assay procedures carried out in dogs where the interval between successive injections is less than 2 hours are misleading unless the phenomenon of tolerance is taken into consideration.