hemagglutinin titer.

Freshly removed lungs from 30 normal Swiss mice, 20 mice infected with Influenza A and 10 mice infected with Influenza B have failed to cause agglutination of mouse red cells. Lung extracts prepared from normal Swiss mice have also given negative results.

Summary. A pneumonitis virus has been isolated during the serial passage of human lung material through Swiss mice. This virus is closely related to the mouse pneumonia virus of Horsfall. The lungs of mice infected with this virus contain an agent capable of agglutinating murine erythrocytes. This agglutination is inhibited by specific anti-sera.

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Observations on Alloxan Diabetes.

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Since the production of necrosis of the islands of Langerhans by alloxan was first reported by Dunn, Sheehan, and McLetchie,[1] permanent diabetes has been produced in rabbits by Bailey and Bailey,[2] in rats by Dunn and McLetchie,[3] and in dogs by Goldner and Gomori.[4] The literature on this subject has been summarized by Joslin[5] and by Bailey, Bailey, and Leech.[6] From our observations confirming the production of alloxan diabetes, we have tried to select those which supplement the data in the literature.

Methods. Normal male rabbits were used and were permitted to eat up to the time of injection. A 2 or 5% aqueous solution of alloxan was injected into the ear vein either in a single dose or in 2 or 3 divided doses 15 to 30 minutes apart. Other routes of administration were tried unsuccessfully. During the next 12 hours some of the animals were given varying quantities of 50% glucose by vein as recommended by Bailey and Bailey.[2] Others were furnished an ample supply of food. In most cases these rabbits ate enough to combat the hypoglycemic stage of alloxan poisoning first observed by Jacobs.[7] Hyperglycemia and glycosuria were noted on the following day and generally reached their greatest severity by the 3rd or 4th day after injection. Once established, the diabetes has persisted throughout the period of observation (maximum 9 months).

All animals were kept in metabolism cages and daily urine collections were made, thymol being used as a preservative. The diet consisted of weighed amounts of cabbage, carrots, and oats. The available carbohydrate of the diet was calculated from standard tables, but no attempt was made to estimate the calories available from cellulose. According to Lusk[8] 22% of cellulose may be digested by the rabbit. However, the figures given for fiber content of cabbage and carrots is 1% so that there would be a relatively small correction, approximately 2% of the usual value for available carbohydrate.

Others have observed that effective doses of alloxan were generally fatal to animals under barbiturate anesthesia. The few dogs,

1 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet*, 1943, **1**, 484.

2 Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

3 Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

4 Goldner, M. G., and Gomori, G., *Endocrinology*, 1943, **33**, 297.

5 Joslin, E. P., *New Eng. J. Med.*, 1944, **230**, 425.

6 Bailey, C. C., Bailey, O. T., and Leech, R. S., *New Eng. J. Med.*, 1944, **230**, 533.

7 Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

8 Lusk, G., *Science of Nutrition*, W. B. Saunders Co., Philadelphia, 1931.

TABLE I.
Incidence and Duration of Alloxan Diabetes in Rabbits.

No. of rabbits	Rabbit No.	Dose of alloxan, mg per kg	Duration of diabetes, days
15		200	0-4
12		200	7-280
	28	25-175 (7 days)	107
	32	50, 0, 100	48
	33	25, 50, 50, 50	101
	35	25-75 (16 days)	72
	36	25 (10 days), 0 (4 days), 100	73
	39	50, 50, 50, 50	34
	40	25, 25, 25, 25	not diabetic
	41	25, 25, 25, 25	" "

cats, and rabbits injected with alloxan intravenously under pentobarbital anesthesia in this laboratory all died. However, it was possible to give full doses (200 mg per kg) to rabbits under light ether anesthesia with no increase in the mortality rate.

Results. Diabetes has been produced by various doses of alloxan (Table I). When 200 mg per kg was given only 12 of 27 rabbits survived more than 4 days. The repeated daily injection of smaller doses was less lethal but 2 deaths in this series are not tabulated. Hard and Carr[9] using single doses of 50 mg per kg of alloxan made one of 5 rabbits diabetic. When they used 100 mg per kg, 8 of 13 rabbits became diabetic, and our results are in good agreement with theirs.

It is clear that the effective dose per day can be lowered somewhat by giving the drug for several days. Rabbits 33 and 39 given 50 mg per kg per day resemble the 3 rabbits made diabetic with 40 mg per kg per day by Bailey *et al.*[6] Hard and Carr[9] state that a refractory animal usually does not respond to successive doses of alloxan, a phenomenon observed in this laboratory also. Goldner and Gomori[4] note that dogs treated with an ineffective first dose show an increased resistance to alloxan. Whatever causes an occasional animal to be refractory, this failure to respond has not been induced in rabbits by repeated small or ascending doses. There are recognized differences in the susceptibility of different animals and of different species[10] to alloxan, but in a single animal it appears that a minimal effective concentration of the drug can cause progressive damage which after several days leads to manifest diabetes. One cannot exclude the possibility that alloxan is destroyed or excreted slowly so that it has a cumulative action, but this seems unlikely when the results of its injection by other routes are considered. It is suggested that it is not the accumulation of the drug but the extension of the pancreatic injury which accounts for this effect.

Alloxan in doses up to 200 mg per kg subcutaneously and in amounts of 300 and 700 (2 rabbits) mg per kg intraperitoneally has caused no evidence of diabetes, renal injury or anemia. It seems that in the rabbit, alloxan is ineffective except by the intravenous route, a finding which suggests that it is rapidly altered when in contact with the tissues. However, in the rat diabetes has been produced by alloxan administered subcutaneously or intraperitoneally[3,11] and the stability of this compound in the body has not yet been studied.

Observations on the Mechanism of Alloxan Action. Table II shows that insulin mixed with 2% alloxan *in vitro* is not inactivated. These results emphasize those of Goldner and Gomori[12] because the smaller dose of insulin (0.2 U per kg) used was still effective.

In order to show that alloxan damages the cells of the islands of Langerhans and that the subsequent liberation of insulin produces the

9 Hard, W. L., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 214.

10 Brunschwig, A., and Allen, J. G., *Cancer Research*, 1944, **4**, 45.

11 Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 287.

12 Goldner, M. G., and Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 73.

TABLE II.
Response to Insulin (0.2 units per kg intravenously) in 2% Alloxan.

	Blood sugar						
		Min					
	Before mg/100 cc	15 mg/100 cc	30 mg/100 cc	45 mg/100 cc	60 mg/100 cc	Fall* %	Alloxan, mg/kg
			Insulin in Alloxan—6 tests.				
Avg	112	101	74	86	84	38	7
Range	100-129	84-114	61-84	69-107	72-117	29-53	
			Insulin alone—48 tests.[17]				
Avg	100	82	60	65	68	44	0

* The difference between the initial blood sugar and the lowest blood sugar observed during each test has been expressed as the percent fall from the initial level.

TABLE III.
Response of Blood Sugar to Alloxan in Normal and Diabetic Rabbits.

			Blood sugar					
				Hr after injection				
Rabbit No.	Condition	Dose of Alloxan (intravenous) mg/kg	Before inj. mg/100 cc	2 mg/100 cc	4 mg/100 cc	6 mg/100 cc	8 mg/100 cc	Ref.
42	Normal	200	114	201	174	56	72	
43		200	108	196	172	42	53	
		200	95	170	80	70	32	(18)
		210	95		50	30	—	(7)
		214	112		205	90	32	(7)
		200	108	237	80	28	—	(12)
		200	102	378	57	40	—	(12)
		150	123	306	—	32		(12)
Avg			107	248	116	48	47	
19	Diabetic fasted	None	510	432	394	362	397	
19			361		353		361	
28			417				318	
32			460				492	
33			285				183	
33			372		361		268	
36			514		430		441	
Avg			417		384		351	
5	Diabetic fasted	200	343	367	364	293	252	
19		200	426			426	397	
28		200	351	362	373	341		
32		200	380	361	352	308	299	
33		200	305	271	234	202	197	
33		200	432			397	374	
36		200	362			435	397	
Avg			371	340	330	343	319	

hypoglycemic phase, alloxan has been given to rabbits with well established alloxan diabetes.* Table III presents these experiments. The control studies included the demonstration of the hypoglycemic effect of 200 mg per kg of alloxan in normal fasted animals. Normal rabbits 42 and 43 agreed with the results of this dosage found in the literature, which have been included for completeness. The figures cited from Jacobs[7] were estimated from his curves. When diabetic rabbits were fasted

[17] Dohan, F. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 24.

* Ridout, Ham, and Wrenshall have just reported 2 such experiments in dogs which yielded similar results. (*Science*, 1944, **100**, 57.)

TABLE IV.
Average Fasting Urinary Excretion of Glucose and Nitrogen.

No. rabbits	Procedure	Urine glucose, g/kg/day	Urine nitrogen	
			Avg, g/kg /day	Range, g/kg/day
5*	Normal	0	0.56 ± 0.11	0.35-1.0
	" 13	0	0.60†	
	" 14	0	0.48†	
7	Alloxan Diab.	1.1	0.78 ± 0.20	0.32-1.80
12‡	Phlorhizin[16]	2.3 ± 0.13	0.77 ± 0.05	0.30-1.20

* The average daily excretion of 4-day fasting periods and S. D. of mean.
† Calculated from statement in text; data not reported.
‡ The averages were calculated from 24 daily determinations on 12 rabbits fasted 1-4 days.

TABLE V.
Effect of Fasting on Blood Sugar of Rabbits.

No. rabbits	Blood sugar			
	Beginning of fast		End of fast	
	Avg mg/100 cc	Range mg/100 cc	Avg mg/100 cc	Range mg/100 cc
5 Normal	113	99-126	109	98-124
7 Diabetic	367	314-536	137	85-202

the blood sugar fell appreciably in the course of 8 hours as shown in the second group of experiments. In diabetic animals given alloxan there was no greater fall than when such rabbits were simply fasted. This adds another type of experiment which supports the concept that the hypoglycemia of alloxan is due to the liberation of pancreatic insulin. In addition, the daily glycosuria of these animals did not increase after the second dose of alloxan.

Metabolic Studies. Little is known about diabetes in the rabbit and the fasting metabolism of this species has not been reported. Table IV presents our findings on fasted diabetic rabbits and includes the data encountered in the literature. The nitrogen excretion of fasted normal rabbits in this study agrees with the statements in the text made by Greeley and Drury[13] and Drury.[14] Greeley[15] described total pancreatectomy in the rabbit but reported no metabolic studies by which it could be compared with other types of diabetes or with other species. In alloxan diabetes, the low excretion of glucose was due to the fact that all but one animal became free of glycosuria before the fourth day of fasting. This improvement was also measured by the fall in blood sugar (Table V) during 4-day fasting periods. The average nitrogen excretion of diabetic animals was not strikingly increased above the normal level. Lusk's[16] data on phlorhizin glycosuria in rabbits provide an interesting comparison. The glycosuria is distinctly greater, the nitrogen excretion about the same as in alloxan diabetes. The significance of these findings will be discussed later.

One observation about the excretion of nitrogen by the rabbit deserves mention. We have found that during a 4-day fast, both normal and diabetic animals, like Lusk's phlorhizinized rabbits, showed great daily irregularities of nitrogen excretion. This exceeds the fluctuations of other species such as the dog, cat, pig, and goat and was not corrected by daily emptying of the bladder by

[13] Greeley, P. O., and Drury, D. R., *Am. J. Physiol.*, 1940, **130**, 249.

[14] Drury, D. R., *J. Clin. Endocrinology*, 1942, **2**, 421.

[15] Greeley, P. O., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 309.

[16] Lusk, G., *Z. f. Biol.*, 1898, **36**, 82.

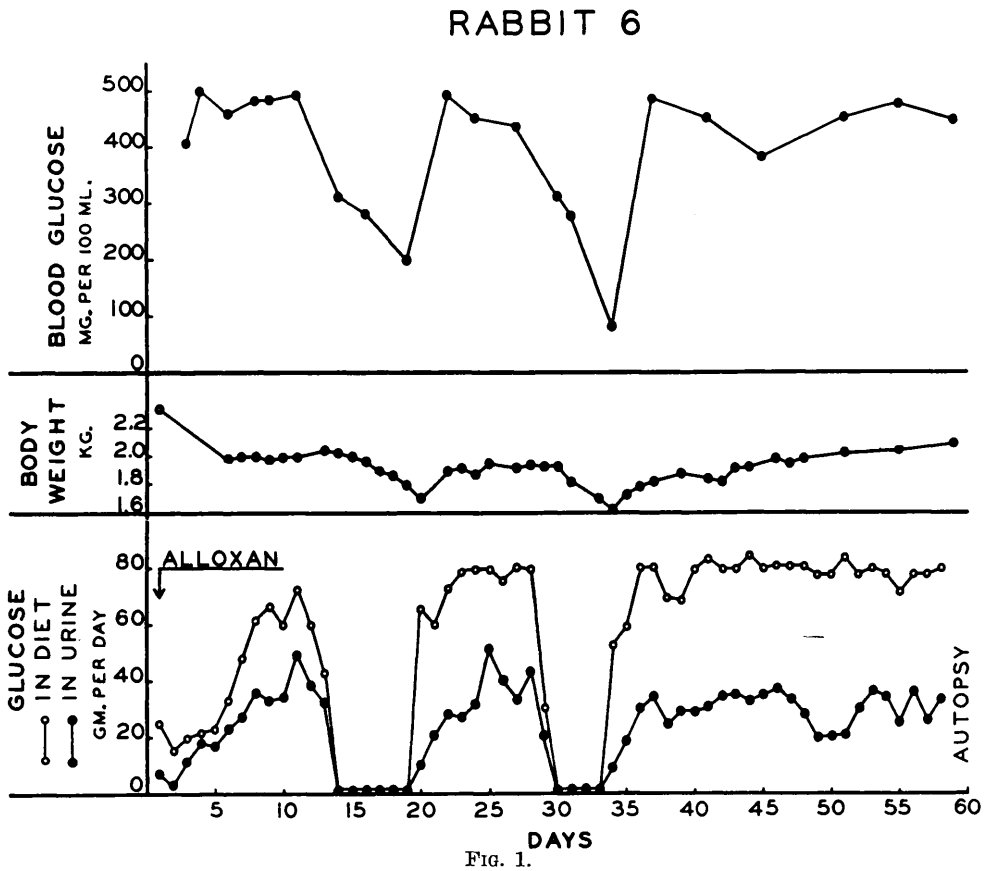

FIG. 1.

Course and severity of diabetes after 200 mg per kg of alloxan had been given on the first day charted. The slight glycosuria on this day was attributed to intravenous glucose. The periods when glycosuria ceased were due to fasting. Note capacity to gain weight in the last days of observation. No insulin was given.

pressure. This behavior of the urinary nitrogen excretion makes the rabbit a relatively poor animal for metabolic studies.

The proportion of the available dietary glucose excreted in the urine has also been used as an index of the severity of the diabetes. Our animals have excreted from 15 to 60% of the available glucose of the diet, the majority being about 40-50%. Fig. 1 illustrates the relationship between food intake and glycosuria in a typical case. The decline of glycosuria during fasting is also noted. About half of the rabbits occasionally had slight ketonuria. In 4 rabbits the diabetes was controlled by insulin for periods from 15 to 27 days. There was no evidence of improvement in the diabetes as a result of this control.

Other Observations. The incidence of early necrosis and subsequent atrophy of the islands and the occurrence of renal damage are summarized in Table VI and need no comment. However, hydropic degeneration has been seen in 2 rabbits examined after diabetes of 48 and 58 days duration. At this time the hydropic change was associated with atrophy and this differs from the hydropic islands described early in the disease by Bailey *et al.*[6] Three animals with prolonged diabetes had normal levels of blood urea nitrogen; 2 which died in 7 and 10 days had elevated values. During the first few days after alloxan the specific gravity of the urine was low (1015) but it returned to normal (1025 to 1055) in 6 animals tested during the chronic stage of diabetes. These changes have not been clearly related to the usually minor pathological alterations in the kidneys.

TABLE VI.
Lesions of Alloxan Diabetes in Rabbits.

Days after alloxan	No. of rabbits	Pancreas			Renal tubules		
		Necrosis	Atrophy	Hydropic	Necrosis	Atrophy	Normal
0-7	7	7	1	0	6	1	0
8-86	12	1	12	2	2	5	5

Hemoglobinemia and hemoglobinuria were observed in a few of our rabbits (*cf.* [1]). Occasional hemoglobin and erythrocyte counts showed that the red blood cells fell to 1 to 2 million a few days after a single large dose and the blood count became normal in about 3 weeks. In the few animals with blood counts, diabetes has not been produced except by a dose of alloxan sufficient to cause anemia.

No sign of cataract formation has been found in 11 rabbits which were diabetic for 6 weeks or longer. This is in contrast to the observation of Bailey *et al.*[6] that cataracts developed in 4 to 6 weeks in rabbits with alloxan diabetes.

Finally, attempts to produce diabetes with nitrates, methylene blue, and sodium tartrate have been entirely negative, the last in spite of its severe action on the renal tubules which is not unlike the milder effect of alloxan.

Discussion. In connection with the results shown in Tables II and III, the findings relevant to the mechanism of action of alloxan may be reviewed. (a) There is transitory hyperglycemia followed by hypoglycemia at 6-12 hours which in turn is followed by diabetes. (b) Alloxan does not inactivate insulin *in vitro*. (c) Although the initial hyperglycemia can be prevented by insulin or phlorhizin, this does not protect the island from necrosis.[12] (d) There is a quantitative similarity between the hypoglycemic effect of alloxan and that of a dose of insulin equivalent to the insulin content of the pancreas.[18] (e) Necrosis of the islands may be apparent in 12 hours or less. (f) There is an absence of the hypoglycemic phase and of any change in the severity of the diabetes, when alloxan is given to diabetic rabbits. All the findings indicate that alloxan injures the cells of the islands of Langerhans with the resultant liberation of insulin which then causes hypoglycemia. The irreversibly damaged cells fail to secrete insulin and diabetes ensues. Alloxan might cause hypoglycemia by temporary injury to the liver. However, its failure to do so in diabetic animals would seem to exclude such an action and to support the hypothesis that damage of the islands is largely, if not entirely, responsible for the results.

The characteristic lesion of alloxan diabetes is atrophy of the islands of Langerhans. The beta cells are particularly vulnerable and a few alpha cells usually remain.[4,19] It is noteworthy that the acute necrosis caused by alloxan and the slower destruction of the beta cells in pituitary-diabetes[20] lead to an essentially similar end result. There has been no recovery from alloxan diabetes treated by insulin,[2,6] a fact which is in agreement with all previous experience with atrophy of the islands.[20] The occurrence of hydropic degeneration in conjunction with island injury appears to be comparable to its development after partial pancreatectomy,[21] and in pituitary-diabetes. Based on previous work, we interpret this finding as indicating that enough beta cells were destroyed by alloxan to make the animals diabetic. The surviving beta cells subsequently became hydropic because of the hyperglycemia. Hydropic degeneration is not seen more frequently (a) because almost all of the beta cells are usually destroyed by alloxan or (b) because of the time when the islands have been examined for this transitory lesion. The occurrence of hydropic degeneration may account for the one dog in which recovery from alloxan dia-

[18] Hughes, H., Ware, L. L., and Young, F. G., *Lancet*, 1944, **1**, 148.

[19] Dunn, J. S., Kirkpatrick, J., McLetchie, N. G. B., and Telfer, S. V., *J. Path. and Bact.*, 1943, **55**, 245.

[20] Lukens, F. D. W., and Dohan, F. C., *Endocrinology*, 1942, **30**, 175.

[21] Allen, F. M., *J. Metab. Res.*, 1922, **1**, 5.

betes was reported by Carrasco-Formiguera.[22]

The metabolism of diabetic rabbits requires comment. When fed they excrete only a moderate amount of carbohydrate. On fasting the glycosuria ceases, there is no ketonuria, and the nitrogen excretion is somewhat greater than that of normal fasted rabbits. These facts are compatible with partial pancreatic diabetes, and the persistence of some islet tissue makes this a probable explanation. However, pancreatic diabetes as seen in the goat[23] prompts one to ask whether alloxan may not produce a severe diabetes similar to that following pancreatectomy. In depancreatized goats there is no glycosuria or ketonuria on fasting and little or none when they are fed an ample amount of greens. After pancreatectomy or phlorhizin the nitrogen excretion is approximately equal in the goat and it is the same after alloxan or phlorhizin in the rabbit. It is thus possible that the seemingly mild diabetes of the rabbit may at times be severe diabetes for this species.

Summary. Diabetes has been produced in rabbits by the intravenous injection of alloxan.[1,2] The effectiveness of the repeated daily injections of doses as low as 50 mg per kg indicates that alloxan has a cumulative action or that a gradual extension of pancreatic injury takes place.

When alloxan was given to rabbits with alloxan diabetes no effect on the blood sugar was observed. This supports the favored hypothesis that the hypoglycemic phase of alloxan poisoning is due to the liberation of insulin from damaged cells.

Metabolic studies indicate that alloxan diabetes in rabbits corresponds to partial pancreatic diabetes. In this connection the factor of species is discussed.

Hydropic degeneration was observed in the islet tissue of 2 animals. This change has been attributed to the effect of hyperglycemia on beta cells which escaped destruction by alloxan.

22 Carrasco-Formiguera, R., *J. Lab. and Clin. Med.*, 1944, **29**, 510.

23 Lukens, F. D. W., *Am. J. Physiol.*, 1938, **122**, 729.

14734

Bacteriostatic Action of Sulfonamide-Penicillin and Urea-Penicillin Mixtures *in vitro*.

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The effectiveness of penicillin* in the treatment of certain types of infections has become well established. In some instances, however, particularly general sepsis and local wound infections, it is possible that combinations of penicillin with other chemo-therapeutic agents will prevent unsuccessful results that are occasionally encountered with penicillin alone. The situation is analogous to that of the sulfonamides, in which urea-sulfonamide mixtures are highly effective for topical application to infected wounds.

The following experiments were designed to study the bacteriostatic action of sulfonamide-penicillin and urea-penicillin mixtures *in vitro*.

Method. Growth curves were measured turbidimetrically under rigidly controlled conditions for a period of 24 hours, according to a method developed in this laboratory and described elsewhere in detail.[1] In the present studies the organisms were a coagulase-positive strain of *Staphylococcus aureus*, and a

* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

1 Kirby, W. M. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.