ings and to determine if salivary glands are selective in their failure to transfer (excrete or secrete) fluoride in contrast to the placenta. as well as to learn more about the biological availability of fluoride in saliva.

*Conclusions.* When pregnant rats receive 50 ppm F/day during entire pregnancy, their pups' molar teeth show a significant reduction in acid solubility. If fluoride administration is continued during lactation, no added anti-solubility effect is shown. Saliva either inactivates fluoride or salivary tissue is selective against fluoride secretion.

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## A Hypoglycemic Factor in Leukemic Tumors. (25684)

MURRAY N. SILVERSTEIN, KHALIL G. WAKIM, ROBERT C. BAHN AND EDWIN D. BAYRD
*Mayo Clinic and Mayo Fn.,* Rochester, Minn.*

The syndrome of fibrogenic tumors and hypoglycemia is becoming more frequently recognized(1). Hypoglycemia was observed in rabbits inoculated with a homogenate of spindle cell sarcoma from rats(2); in mice with leukemic tumors(3); and in mice bearing transplantable pituitary tumors(4). We observed earlier considerable amelioration of alloxan diabetes in AKR mice inoculated with lymphatic leukemia tumor BW5147. Comparison of progression of growth of tumor with onset and magnitude of decrease in blood sugar in normal and diabetic animals suggested that increase in the mass of tumor alone did not explain hypoglycemia in these mice. Further studies employing $C^{14}$ glucose revealed no excessive avidity of these tumors for glucose. The pancreas and adrenal glands from normal animals with tumor and from diabetic animals with and without tumor were studied. No islet cell hypertrophy or hyperplasia in normal animals with tumor was observed. The pancreas of tumor-bearing animals and of normal animals revealed equally severe damage to islet cells after administration of alloxan. Furthermore, as diabetes in tumor-bearing animals was ameliorated the pancreas did not show regeneration of islet cells. Adrenal glands of diabetic animals with and without tumor were similar. These findings suggested possible presence of a hypoglycemic factor in this tumor.

*Methods.* AKR mice and the tumor BW5147 of lymphatic leukemia were obtained from Jackson Memorial Lab., Bar Harbor, Me. Three types of extracts were prepared from 15-day-old tumors. At this age of tumors, mice exhibited profound hypoglycemia and tumors were large with little gross evidence of necrosis. All animals were initially inoculated subcutaneously into the right thigh with 50 mg of tumor extract.† The tumor was homogenized in Waring blendor with 4 ml of 0.9% solution of sodium chloride/g of tumor. The homogenate was refrigerated 48 hours, centrifuged and the supernatant decanted. The fluid was evaporated in a watch glass in air at room temperature to concentration of 1 g of tumor/0.5 ml of saline solution. A second extract was made as described by Scott and Fisher(5) for extraction of insulin. The final material for assay was reconstituted in saline at pH 2.5 so that the material extracted by insulin procedure from 1 g of tumor would be present in 0.5 ml of the acid saline solution. For the third extract the

* The Mayo Fn., Rochester, Minn., is part of Graduate School, Univ. of Minnesota.

† Silverstein, M. N., Wakim, K. G., Bahn, R. C., Bayrd, E. D., unpublished data.

TABLE I. Saline Extract of Tumor in 8 Mice.*

| Blood sugar, mg/100 ml | | |
|---|---|---|
| Before inj. | 1 hr after inj. | % change |
| 293 | 283 | − 3.4 |
| 290 | 340 | +17.2 |
| 370 | 347 | − 6.2 |
| 453 | 417 | − 7.9 |
| 447 | 420 | − 6.0 |
| 450 | 430 | − 4.4 |
| 445 | 477 | + 7.2 |
| 410 | 420 | + 2.4 |
| | Mean | − 0.14 |

* Intrav. dose of 0.5 ml of extract contained 1 g of tumor.

tumor was homogenized with 5 ml acetone (triple distilled in glass)/g. After refrigeration for 48 hours, the mixture was centrifuged 30 minutes, and the supernatant acetone was decanted and transferred to a watch glass for evaporation as before. The material on the watch glass was suspended in 0.9% sodium chloride adjusted to pH 2.5 with hydrochloric acid. Final concentration was so prepared that the acetone-extractable material from 1 g of tumor would be present in 0.5 ml of acid saline solution. Acetone extracts of tumors from animals with alloxan diabetes were compared in potency with similar extracts of tumors from normal animals. Alloxan diabetic AKR mice used as assay animals were lightly anesthetized with nembutal (0.75 μg/20 g mouse given intraperitoneally) and a fresh 1% solution of alloxan monohydrate was administered rapidly by tail vein. Each mouse received 1 mg of alloxan monohydrate/10 g of body weight and was used for assay after 48 hours. For assay the alloxan diabetic mouse was anesthetized with nembutal and 0.12 ml of blood was removed by cardiac puncture. In one group of mice the saline extract was given intravenously slowly (into tail vein) in one dose of 0.5 ml corresponding to 1 g of tumor. In another group the acetone extract was given in same manner, and blood was drawn from heart 1 hour later. Since use of intravenous route caused death, insulin extractable material was injected intraperitoneally. Blood was withdrawn by cardiac puncture either 1 or 2 hours later. True blood sugar was determined by microtechnic on a modified Somogyi filtrate.‡

‡ Method devised by Dr. Warren F. McGuckin, Sect. of Biochem., Mayo Clinic.

*Results.* After administration of saline extract of tumor, the mean change in 8 animals, Table I, was a fall in blood sugar level of 1.13% with ranges from a rise of 17.3% to a fall of 7.9%.

With material obtained by insulin-extraction procedures, the mean change for the 8 animals was a rise in blood sugar, Table II, of 5.5% with ranges from a rise of 22.1% to a fall of 9.3%.

After intravenous administration of acetone extract, all 11 animals showed, Table III, a moderate to severe fall in blood sugar, with mean change of 24.1% with ranges of 19.0% to 30.8% decrease.

TABLE II. Tumor Extract by Insulin-Extraction Procedure in 8 Mice.*

| Blood sugar, mg/100 ml | | |
|---|---|---|
| Before inj. | 1 hr after inj. | % change |
| 460 | 470 | + 2.2 |
| 430 | 465 | + 8.1 |
| 557 | 680 | +22.1 |
| 283 | 287 | + 1.4 |
| 530 | 620† | +17.0 |
| 474 | 430† | − 9.3 |
| 500 | 480† | − 4.0 |
| 407 | 410† | − 0.7 |
| | Mean | + 4.6 |

* Intraper. dose of 0.5 ml of extract contained 1 g of tumor.
† Two hr after inj.

The illustration shows time-response curve after administration of acetone extract as described. A fall in blood sugar level of 7% occurred at 15 minutes, of 30% at 30 minutes, of 25% at 1 hour, and of 7% at 2 hours.

TABLE III. Acetone Extract of Tumor in 11 Mice.*

| Blood sugar, mg/100 ml | | |
|---|---|---|
| Before inj. | 1 hr after inj. | % change |
| 620 | 443 | −28.5 |
| 520 | 417 | −19.8 |
| 603 | 443 | −26.5 |
| 543 | 440 | −19.0 |
| 447 | 321 | −28.2 |
| 467 | 323 | −30.8 |
| 447 | 340 | −23.9 |
| 490 | 365 | −25.5 |
| 489 | 372 | −23.9 |
| 540 | 433 | −19.8 |
| 523 | 420 | −19.7 |
| | Mean | −24.1 |

* Intrav. dose of 0.5 ml of extract contained 1 g of tumor.

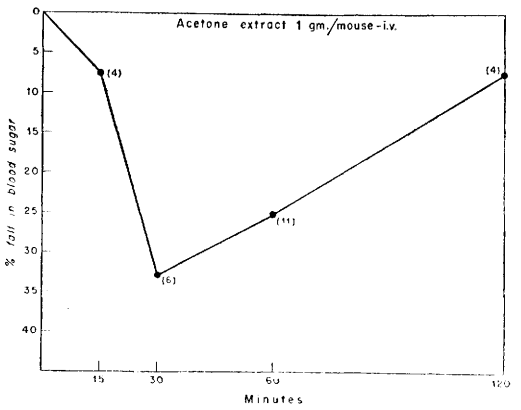


FIG. 1. Time-response curve.

When only acid saline solution at pH 2.5 was injected intravenously in doses of 0.5 ml. a mean rise of blood sugar of 1.5% was observed, Table IV, with ranges of 12.6% rise to an 8.9% fall.

Extracts of tumor obtained from diabetic animals did not produce significantly different hypoglycemia from extracts of tumors of nondiabetic animals. Another interesting finding was the gradual diminution in extract potency with time.

*Comment.* Simple acetone extracts of lymphatic leukemia tumor BW5147 caused moderate hypoglycemic effects when given intravenously to alloxan diabetic AKR mice. Material obtained by insulin extraction procedures, however, produced negative results by the intraperitoneal route. In human cases of fibrogenic tumors with hypoglycemia, only one tumor produced insulin-like activity(6).

The nature of the hypoglycemic factor in tumors is questioned. Procedures for extraction of insulin indicate that this factor might not be insulin. The question arises, could it be another protein moiety or a lipide? Studies are in progress to determine the nature of the factor.

It would be of interest to determine if many different types of malignant tumors contain an acetone extractable hypoglycemic agent.

*Summary.* A fall in blood sugar was observed in AKR mice inoculated with leukemic tumor BW5147. Marked amelioration of diabetes resulted from inoculation of alloxanized AKR mice with lymphatic leukemic tumor. Growth of this tumor was studied in normal and diabetic mice. Rate of tumor growth and blood sugar values were correlated. Increase in mass of tumor alone did not seem to explain hypoglycemia in these mice. Consequently, 3 extracts of tumor were made and tested for presence of a hypoglycemic factor. Blood sugar values of diabetic mice were determined before and after injection of tumor extracts. A saline extract did not show a hypoglycemic effect, nor did an extract made by insulin-extraction procedure. An acetone extract, however, exhibited moderate hypoglycemic activity. Time-response curves demonstrated that acetone-extractable material derived from 1 g of fresh tumor produced a fall in blood sugar of 7% at 15 minutes, 30% at 30 minutes, 25% at 1 hour, and 7% at 2 hours.

TABLE IV. Acid Saline Controls in 8 Mice.*

| Blood sugar, mg/100 ml | | |
|---|---|---|
| Before inj. | 1 hr after inj. | % change |
| 550 | 570 | + 3.6 |
| 620 | 600 | - 3.3 |
| 364 | 410 | +12.6 |
| 358 | 344 | - 3.9 |
| 373 | 340 | - 8.9 |
| 490 | 512 | + 4.5 |
| 410 | 407 | - 0.7 |
| 500 | 538 | + 7.6 |
| | Mean | + 1.5 |

* Dose of 0.5 ml of 0.9% sodium chloride given intrav.

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