pounds (*2*, *3*) or in mice and rats (*8*) administered, orally, the potent urinary bladder carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. No other tissues of mice exposed to sodium cyclamate exhibited a tumor incidence significantly different from that of the control mice.

It was only after this demonstration of the carcinogenic activity of sodium cyclamate for mouse bladder that attention was drawn to the urinary bladder as an organ susceptible to the carcinogenicity of this compound. Indeed, when the bladders of rats given cyclamate orally were examined, infiltrating carcinomas were observed (*9*), and bladder carcinogenicity of the cyclamate demonstrated by the pellet implantation technique was confirmed. Thus we conclude that this technique is sensitive, reproducible, and predictive of the bladder carcinogenicity of a chemical administered by means other than direct bladder exposure. It is a valid method of assessing carcinogenic activity, a conclusion shared by other authorities (*10*).

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References and Notes

1. R. A. Ballinger, *U.S. Dep. Agr. Agr. Econ. Rep. No. 133* (U.S. Government Printing Office, Washington, D.C., 1967).
2. G. T. Bryan, *Amer. Ind. Hyg. Ass. J.* **30**, 27 (1969).
3. ———, R. R. Brown, J. M. Price, *Ann. N.Y. Acad. Sci.* **108**, 924 (1963); *Cancer Res.* **24**, 582 (1964); G. T. Bryan, R. R. Brown, C. R. Morris, J. M. Price, *ibid.*, p. 586; G. T. Bryan, R. R. Brown, J. M. Price, *ibid.*, p. 596; G. T. Bryan and P. D. Springberg, *ibid.* **26**, 105 (1966); G. T. Bryan, *J. Nat. Cancer Inst.* **43**, 255 (1969).
4. Obtained as a gift of analytically chemically pure sodium cyclamate (lot No. 785-7021) from Abbott Laboratories, North Chicago, Ill.
5. Performed under supervision of Dr. R. G. Wiegand, Abbott Laboratories.
6. G. M. Bonser and J. W. Jull, *J. Pathol. Bacteriol.* **72**, 489 (1956); F. J. C. Roe, *Brit. J. Urol.* **34**, 238 (1964).
7. R. A. Fisher, *Statistical Methods for Research Workers* (Hafner, New York, ed. 13, 1958), pp. 96–97.
8. E. Ertürk, J. M. Price, J. E. Morris, S. M. Cohen, R. S. Leith, A. M. Von Esch, A. J. Crovetti, *Cancer Res.* **27**, 1998 (1967); E. Ertürk, J. M. Price, G. T. Bryan, *Proc. Amer. Ass. Cancer Res.* **10**, 23 (1969); E. Ertürk, S. M. Cohen, J. M. Price, G. T. Bryan, *Cancer Res.*, in press; E. Ertürk, S. M. Cohen, G. T. Bryan, *ibid.*, in press.
9. Anonymous, *Med. World News* **10**, 4 (1969); J. M. Price, C. G. Biava, B. L. Oser, E. E. Vogin, J. Steinfeld, H. L. Ley, *Science*, in press.
10. G. M. Bonser, E. Boyland, E. R. Busby, D. B. Clayson, P. L. Grover, J. W. Jull, *Brit. J. Cancer* **17**, 127 (1963); E. Boyland, E. R. Busby, C. E. Dukes, P. L. Grover, D. Manson, *ibid.* **18**, 575 (1964); D. B. Clayson, *Can. Cancer Conf.* **6**, 186 (1966); A. M. Pamukcu, C. Olson, J. M. Price, *Cancer Res.* **26**, 1745 (1966); D. B. Clayson *et al.*, *Brit. J. Cancer* **22**, 825 (1968).
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Proinsulin: Metabolic Effects in the Human Forearm

Abstract. *Five subjects were studied by the forearm perfusion technique with proinsulin at a final plasma concentration of 1.76×10^{-9} millimole per milliliter. Two of these subjects were studied with single-component insulin at a concentration of 1.96×10^{-9} millimole per milliliter. Proinsulin is, in general, biologically less potent than single-component insulin. In contrast to insulin, its effects upon adipose tissue are for the most part greater than upon muscle.*

Proinsulin, a single chain polypeptide, is the precursor of insulin in man and other animal species (*1*). The metabolic effects of physiologic concentrations of proinsulin and of insulin free of both proinsulin and glucagon have not been investigated. With the human forearm as a test system, the effects of proinsulin and insulin (*2*) were studied on peripheral fat and muscle tissue. By using a double lumen arterial needle placed in the brachial artery we were able to sample incoming blood (proximal tip) and constantly infuse Evans Blue or Evans Blue plus hormone distal to the sampling site (*3*). Locally controllable concentrations of the hormones were achieved without significant concentration changes elsewhere. The mean concentration of proinsulin in venous plasma was 1.76×10^{-9} mmole/ml during an infusion while the arterial concentration increased only 0.13×10^{-9} mmole/ml. No change of arterial concentration of insulin could be detected during infusions with single-component insulin. Simultaneous blood samples from the deep venous and superficial beds were representative of muscle and adipose tissue, respecively (*4*).

Samples were analyzed for plasma potassium, free fatty acid (FFA), glucose and total insulin-proinsulin (*5*). The hematocrit was determined for all samples (arterial, deep venous, and superficial venous), and dye concentrations were measured in order to calculate blood flow. Volume in the forearm was measured at the end of the experiment, and from plasma flow and forearm volume measurements, a mean venous transit time (extracellular volume per amount of plasma flow per minute = transit time) was calculated for each subject. Blood flow in the

Table 1. Net mean maximum effects of proinsulin $[(A-V)-V_0$ in which A is the arterial concentration of metabolite and V is concentration in a venous bed. In adipose tissue the venous bed is a superficial venous bed, in muscle tissue the venous bed is a deep venous bed. V_0 is mean arteriovenous concentration difference of a metabolite during a 1-hour control period]. Hormone infusions lasted 30 minutes with hand circulation excluded by a wrist cuff inflated to greater than 200 mm-Hg. Ranges are given in parentheses.

Tissue	FFA (μmole/ml)	K^+ ml) μeq/	Glucose (μmole/ml)
Adipose	.418 (.165–.809)	.218 (.04–.89)	.33 (.19–.79)
Muscle	.389 (.082–.783)	.24 (.06–.55)	.21 (.07–.42)

Table 2. Comparison of net mean maximum effects of proinsulin and single-component insulin. Effect is computed as in Table 1. Percent is computed by dividing the net mean maximum effect of insulin into the net mean maximum effect of proinsulin and then multiplying the quotient by 100.

Tissue	FFA			K^+			Glucose		
	Proinsulin (μmole/ml)	Insulin (μmole/ml)	Effect (%)	Proinsulin (μmole/ml)	Insulin (μmole/ml)	Effect (%)	Proinsulin (μmole/ml)	Insulin (μmole/ml)	Effect (%)
				Subject L.H.					
Adipose	.177	.335	52.8	.10	.34	28.0	.54	.92	59.0
Muscle	.082	.518	15.8	.06	.39	29.0	.41	1.38	30.0
				Subject E.K.					
Adipose	.358	.256	135.0	.04	.33	22.0	.19	.49	39.0
Muscle	.165	.278	59.0	.22	.92	24.0	.07	1.13	6.2

forearm did not change with the infusion of either insulin or proinsulin.

The subjects were five healthy, nonobese males who had no family history of diabetes mellitus. Five proinsulin and two subsequent single-component insulin infusions were carried out in these individuals. The dose of proinsulin was calculated to be equivalent to the maximum molar weight of insulin used in past forearm experiments (100 μU kg^{-1} min^{-1} or 6.4×10^{-10} mmole kg^{-1} min^{-1} (*3*).

From experiments with glucagon-free insulin (300 to 400 μU/ml final plasma concentration) the following metabolic effects have been noted: (i) Insulin blocked lipolysis in adipose tissue and unmasked ongoing uptake of FFA by muscle. (ii) Efflux of K^+ was blocked in muscle, but there was a much lesser effect on adipose tissue. (iii) Glucose uptake was increased up to 12-fold in muscle and 6-fold in adipose tissue (*6*).

In this study, net maximum or net mean maximum alterations from the basal state were used as an index of the hormones' biologic action; that is, arteriovenous concentration differences in a given bed minus the mean basal arteriovenous concentration differences in that bed (Table 1). Positive changes represent an increased uptake of a given substance in comparison with the basal state for that tissue; negative changes represent an output of a substance relative to the basal state.

A comparison of the effect of proinsulin alone with the effect of single-component insulin in equimolar concentrations in the same individuals demonstrated a decreased biologic potency of proinsulin. The relative arteriovenous concentration differences for FFA, K^+ concentration, and glucose were less, with one exception, for proinsulin than insulin. Proinsulin had as little as 6 percent of the effect of insulin upon increasing glucose uptake across the deep bed in one subject (Table 2). In one of the two individuals (E.K.) studied with both insulin and proinsulin, proinsulin had a greater effect than single-component insulin upon differences in arterial and venous amounts of FFA in adipose tissue. This raises the possibility that proinsulin's effect upon differences in arterial and venous concentration of FFA in adipose tissue may be equivalent to or greater than the effect of insulin.

In general, proinsulin had more effect upon the superficial venous bed (predominantly fat) than upon the deep bed (predominantly muscle). This was true for both FFA and glucose, whereas effects on K^+ were approximately equal (Table 1). This pattern of tissue sensitivity contrasts sharply with present and past forearm experiments with insulin (Table 2). These observations may be pertinent to states in man where proinsulin constitutes a greater proportion of total immunoreactive insulin than normal (*7*, *8*).

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References and Notes

1. D. F. Steiner, D. Cunningham, L. Spigelman, B. Atten, *Science* **157**, 697 (1967); D. F. Steiner, O. Hallund, M. B. Rubenstein, S. Cho, C. Bayliss, *Diabetes* **17**, 725 (1968); P. T. Grant and K. B. M. Reid, *Biochem. J.* **109**, 32P (1968); R. E. Chance and R. M. Ellis, *Arch. Int. Med.* **123**, 229 (1969).
2. Porcine proinsulin CT-1649-8A and single-component porcine CT-1651-8A insulin were obtained from Dr. R. Chance (Lilly Research Laboratories). We thank Drs. Chance and J. Galloway of Lilly Laboratories.
3. R. Andres, M. A. Baltzan, G. Cader, K. L. Zierler, *J. Clin. Invest.* **41**, 108 (1962).
4. R. Andres, G. Cader, K. L. Zierler, *ibid.* **35**, 671 (1956); M. A. Baltzan, R. Andres, G. Cader, K. L. Zierler, *ibid.* **41**, 116 (1962); R. Andres, K. L. Zierler, M. Anderson, W. N. Stainsby, G. Cader, A. S. Ghrayyib, J. L. Lillienthal, Jr., *ibid.* **33**, 482 (1954).
5. T. J. Merimee, D. A. Lillicrap, D. Rabinowitz, *Lancet* **1965-II**, 668 (1965).
6. K. L. Zierler and D. Rabinowitz, *Medicine* **42**, 385 (1963).
7. A. H. Rubenstein, S. Cho, D. F. Steiner, *Lancet* **1968-I**, 1353 (1968).
8. R. Gorden and J. Roth, *Arch. Int. Med.* **123**, 237 (1969).
9. Supported in part by PHS training grant AM 054456-04, PHS grant 1 RO1 AM 13565-01, and by a grant from the American Diabetic Association.

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Somatic Association in Avena sativa L.

Abstract. *Root tip cells of hexaploid oats* Avena sativa *L. were examined at mitotic metaphase, and distances between homologous as well as between nonhomologous chromosomes were measured and their frequency distributions compared. Nonhomologous chromosomes were scattered at random in the cells studied. In contrast, the mean distance between homologous chromosomes was significantly shorter. There is a tendency for somatic association of homologs in this species.*

Association of homologous chromosomes in somatic cells has been reported for a number of organisms (*1*, *2*). Recently, Feldman *et al.* (*1*) measured the distances between members of pairs of homologous and between nonhomologous chromosomes in root tip cells of common wheat and found that the homologous chromosomes were not distributed at random but were associated with one another. In addition, Feldman (*3*) presented evidence that genes, located on chromosome 5B, 5D, and 5A, regulate this somatic association.

In *Avena sativa* L. a number of chromosomes can be easily identified by karyotype in somatic cells. Thus it is possible to determine whether the phenomenon of somatic association is also present in this species.

For this purpose the distances between members of chromosomes pair 21 and between members of a pair of homologous telocentrics were measured. Chromosome 21 is the shortest of the complement and easily distinguished (Fig. 1). In addition, the distances between the telocentrics and chromosomes 21 were scored. The telocentrics were not identified but were not chromosome 21 since the 21 bivalents always formed at meiosis, the telocentrics pairing together to form an open bivalent. The telocentrics could be differentiated from chromosome 21 by their terminal centromeres in somatic metaphase.

Distances between the midpoints of telocentrics, distances between centromeres of the chromosomes 21, and distances between the midpoints of the telocentrics and the centromeres of the chromosomes 21 were taken in the same cell. The distance between the two chromosomes farthest apart in the cell concerned was also taken to mini-

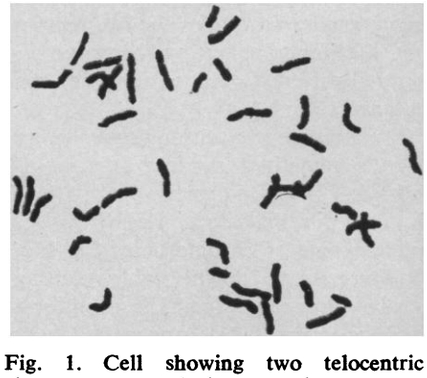

Fig. 1. Cell showing two telocentric chromosomes and the two chromosomes 21 (shortest of the complement).