vals of 24, 48 and 72 hours after injection of the urine extract to determine when maximum uptake of Fe59 by erythroid precursors occurs. This was found to be at 48 hours after initiation of erythropoietic stimulation. It would appear that some escape from the polycythemic suppression of erythropoiesis in the hypertransfused guinea pigs occurs when the assay is prolonged to 72 hours as noted by the increased incorporation of Fe59 into erythrocytes of the 72-hour controls.

The previously reported failure of human erythropoietin to stimulate erythropoiesis in the normal guinea pig could have been due to formation of antibodies to the heterologous erythropoietin, as suggested by Garcia and Schooley(6). The short duration (of some) of these experiments, however, make this explanation unlikely. Another possibility is that the assay animals used were not sensitive enough under the experimental conditions to record an erythropoietic response. However, it might have been postulated that human erythropoietin has a different molecular structure from guinea pig erythropoietin and consequently has a limited ability to activate erythropoiesis in the guinea pig and possibly other species(9). The possible species differences of molecular structure of erythropoietin has not been sufficiently explored because of the difficulties of erythropoietin purification. However, as has been shown in this experiment the hypertransfused guinea pig is able to respond to human erythropoietin. A dose response curve with a standardized erythropoietin preparation must be determined in order to compare the guinea pig's response to that of other species.

Summary. Fe59 RBC incorporation was increased in the hypertransfused guinea pig following administration of an extract of urine from a patient with chloramphenicol-induced hypoplastic anemia. This extract also had erythropoietic activity in the polycythemic mouse. Maximum Fe59 incorporation into erythrocytes of guinea pigs occurred when the Fe59 C13 was given 48 hours after administration of the erythropoietically active human urine extract.

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Dextran-Glucose and the Cultivation of Leucocytes. (29410)

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Arakaki and Sparkes(1) have described a micromethod for cultivation of leucocytes of whole blood which makes possible short term cultures with a small inoculum. However, it requires the addition of serum or plasma enrichment, and thus the medium contains an ingredient which may vary in its content. It would therefore be of advantage if, instead, a more nearly inactive standardized substance could be substituted for the enrichment ingredient. Such a substitute would be particularly desirable if it were inactive either as a mitotic stimulator or inhibitor. A commercial 6% dextran solution containing 5.0% glucose (Abbott) has been found to be at least a partially effective substitute for the

TABLE I. Colchicine Metaphase Index in Cultures of Human Leucocytes.

Nutrient additive		Inoculum			Colchicine metaphase index*	
Plasma enrichment, ml	Dextran, ml	Plasma, ml	Whole blood, ml	Total WBC, millions	I	II
2.5		1.8		22.0	2	4
2.5			.3	1.5	17	21
	3.0		.3	1.5	12	13
	3.0	1.8		22.0	.6	.7
	3.0	3.0		37.0	.3	0

Culture medium contained 5.0 ml TC 199 (Difco) in addition to ingredients indicated above. In addition, each culture contained 0.123 ml of a 1:10 dilution of phytohemagglutinin M (Difco), added last.

* Cultures were run in duplicate and counts made on each. Index is number of metaphase figures per thousand leucocytes.

TABLE II. Colchicine Metaphase Index in Cultures of Guinea Pig Leucocytes.

Nutrient additive		Inoculum			Colchicine metaphase index*	
Plasma, ml	Dextran, ml	Plasma, ml	Whole blood, ml	Total WBC, millions	I	II
4.5			.3	1.8	11	15
	3.0		.3	1.8	11	12
3.0		.8		33.0	5	4

Other ingredients in culture medium as in Table I. * Duplicate cultures as in Table I.

plasma or serum enrichment. The results of a comparison between microcultures with plasma enrichment and dextran-glucose are reported here.

Materials and methods. Thirty successful cultures of human leucocytes were carried out in media containing plasma enrichment and 10 in media with the dextran-glucose substitute. Similarly 30 successful cultures of guinea pig leucocytes were obtained with media containing plasma enrichment and 20 with the dextran-glucose substitute. In all of these the results were approximately comparable. In selected instances the same blood was used as the inoculum, and the comparisons carried out. Two such experiments using the blood of a single normal human female subject are shown in Table I. In the first, small inocula (0.2-0.4 ml of whole blood) were cultured in a medium consisting of 5 ml TC 199 (Difco) and 2.0-4.0 ml of plasma. These were compared with similar cultures in which 2.0-4.0 ml of dextran-glucose was substituted for the plasma. This is basically the micromethod of Arakaki and Sparkes(1). Colchicine treatment, centrifugation, washing and osmotic swelling, methanol-acetic acid fixation, and staining with the conventional Giemsa stain were carried out as described by Moorhead *et al*(2). In the second experiment with blood from the same human female, suspensions of leucocytes in plasma were prepared according to the method of Moorhead *et al*(2) and cultured in the same 2 media as noted above. A similar experiment with the blood of one guinea pig is shown in Table II.

Results. Table I shows the results obtained with the blood of the single human female subject. The first horizontal row shows the results obtained by the macromethod of Moorhead *et al*(2), and the second by the micromethod of Arakaki and Sparkes(1). The results were considerably better with the micromethod and correspond roughly to the 3% reported by Arakaki and Sparkes (1). The third horizontal row shows the metaphase index of a microculture in dextran-glucose medium, and the fourth and fifth macrocultures with the same medium. The index obtained with the dextran-glucose microculture was considerably greater than with the macrocultures containing either plasma enrichment or the dextran-glucose substitute; it was only slightly lower than the microculture containing plasma enrichment.

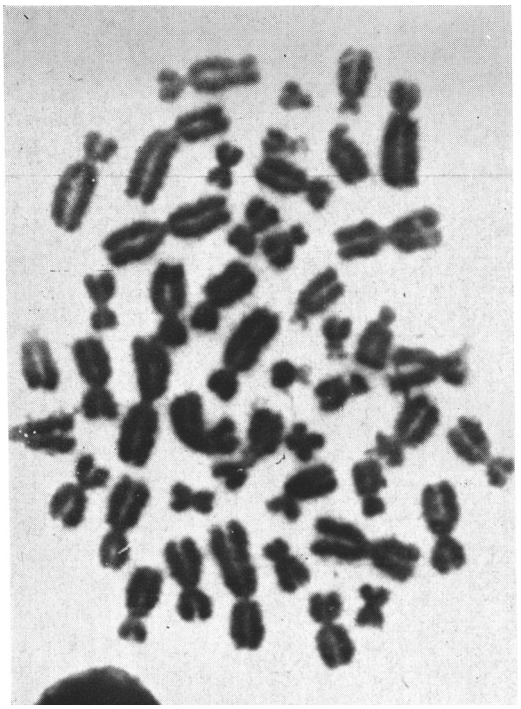

FIG. 1. Colchicine-metaphase of human leucocyte cultured in dextran-glucose medium.

Macrocultures containing plasma enrichment showed a higher metaphase index than similar cultures with the dextran-glucose substitute.

Since the ultimate purpose of such cultures is to study the structural characteristics of individual chromosomes, it is also important to compare the quality of the mitotic figures obtained. Fig. 1 is from a microculture containing dextran-glucose, and shows a good separation and retention of structural characteristics.

Table II shows the results obtained with the blood of a single guinea pig. The first horizontal row shows the results obtained with a microculture containing plasma enrichment and the second with the dextran-glucose substitute. The metaphase index is about the same in both. The third row shows the result of a macroculture containing plasma enrichment in which the metaphase index is lower than that obtained with the two microculture technics. Good separation and retention of structural characteristics of chromosomes were obtained with all 3 types of cultures.

Discussion. This study indicates that both the micromethod of Arakaki and Sparkes and our method in which the dextran-glucose is substituted for plasma enrichment are effective. While there may not be a particular advantage in making the substitution for plasma, there may be occasions when this is desirable.

In an ordinary culture of approximately 8 ml (5 ml TC199 + 3 ml dextran-glucose + 0.3 ml inoculum), it is possible to reduce the content of plasma proteins from about 1.5% to about 0.06% by this substitution. While this is not a zero baseline, it represents a considerable reduction. Dextran may not be a completely inert material since it has been demonstrated that certain fractions of dextran are able to bring about the fixation of complement(3, p. 294). Moreover, aggregates of a granular material were seen on some slides prepared from cultures containing dextran, but these were not found intracellularly. There are also other differences between the dextran glucose medium and those containing plasma or serum. Not only are the protein concentrations altered, but also those of glucose, sodium and chloride. It is not known what the influence on the metaphase index would be if one adjusted these concentrations. Dextran-glucose in solution has a pH of 4.0, but when added to TC199 the pH is 7.2.

This technic is particularly applicable for use with small animals where obtaining large volumes of blood, especially for repeated cultures, may be undesirable. Moreover, in guinea pigs in particular, there is an increased tendency of the plasma inoculum to clot; such clots appear to interfere with the successful development of the culture. The use of small inocula minimizes this interference. As already noted, the use of dextran-glucose as a substitute for plasma enrichment may be particularly advantageous for the study of the influence of various plasma protein fractions and salt concentrations on mitosis. It may also be useful for the study of the effects of a variety of substances which might be inactivated by serum or plasma inhibitors.

Summary. A microculture medium for the culture of white blood cells has been utilized in which dextran-glucose has been substituted for plasma enrichment. The metaphase index obtained with this medium utilizing human and guinea pig blood as the inoculum has been almost as high as with plasma enrichment. It would appear to be particularly useful for the study of the influence of various plasma constituents on cell growth in tissue culture, as well as for the effects of a variety of substances which may be inactivated by serum or plasma inhibitors.

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Glucose Concentration and Insulin Dose-Response Curves Using Rat Fat Tissue *in vitro*.* (29411)

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Very few variables have been described as influencing the sensitivity of the response of rat epididymal fat *in vitro*. These variables have been confined either to variations between animals and between different portions of epididymal fat(1), or to the effect of fasting and refeeding rats before sacrifice (2). The sensitivity of the insulin dose-response curve is here defined as the degree of change in the metameter of response produced by a small increase in low concentrations of insulin, *i.e.*, the steeper the slope of the standard curve, the greater the sensitivity. In these terms, rat epididymal fat pad is currently recognized as the most insulin-sensitive tissue available for *in vitro* studies. Despite the increasing use of the fat pad assay, there appears to be no rational basis for the choice of glucose concentration (200-300 mg%) advocated in the literature. As there appears to be no study of a potentially major variable, glucose concentration *in vitro*, the present study was designed to test insulin sensitivity at glucose concentrations *in vitro* equivalent to hyperglycemia and hypoglycemia in the rat or in man. In addition, a modified assay superior to its original in terms of the index of precision is described, and its value assessed.

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Methods. The basic insulin dose-response method of Renold *et al*(1), measuring $^{14}CO_2$ formation from ^{14}C-1-glucose was used, but the pool design proposed by Froesch *et al* (3) was adopted to minimize the error due to animal variation in any experiment. All experiments were performed in one month from December 19, 1963 to January 21, 1964, a stable period with regard to rat variation in this laboratory. Six Wistar rats, fed *ad lib* on Purina rat chow, differing less than 15 g by weight (range 140-180 g), were chosen for each dose-response curve. Each epididymal fat pad was cut into 6 pieces, so that 12 flasks received a pool of fat from all 6 rats, with an even distribution of pieces from each pair of fat pads(3). The wet weight of fat tissue per flask ranged from 80 to 140 mg in all experiments, and the weights of tissue for determination of each dose-response curve usually agreed within 25 mg. The incubation medium was Krebs-Ringer bicarbonate buffer containing 200 mg% gelatin(1) and varying concentrations of glucose. Each flask contained 2 ml of