Table 1. Comparison of mean body weights (in grams) up to weaning of 129B6-*dy* uniform F_1 hybrid dystrophics, their normal littermates, with 129/Re-*dy* dystrophics and normals.

Age (day)	Genetic background	*dydy* ♀		*Dy-* ♀		*dydy* ♂		*Dy-* ♂	
		No.	Mean wt.	No.	Mean wt.	No.	Mean wt.	No.	Mean wt.
14	129B6 F_1	6	7.3	6	8.4	3	8.3	3	10.3
21	129B6 F_1	5	10.6	4	13.0	6	10.6	5	13.1
28	129B6 F_1	6	15.0	6	17.3	11	15.8	10	19.3
28	129/Re	12	12.4	12	17.6	5	12.6	7	19.4

(94 percent) set aside for longevity studies have survived more than 150 days, and 43/50 (84 percent) have survived 210 days. Their *median* life-span is close to 250 days.

The increased vigor and prolonged life-span of uniform 129B6-*dy* F_1 dystrophics suggests that they may be much more useful animals for many research projects than are the presently used 129/Re-*dy* dystrophics. It should be stressed that although 129/Re-*dy* × C57BL/6J-*dy* F_1 hybrid dystrophics are heterozygous for all genes by which 129/Re and C57BL/6J differ, it is still true that all individuals *within* one F_1 hybrid group are as much like each other as are the members of a single inbred strain. It is also true that 129B6 F_1-*DyDy* and 129B6 F_1-*Dydy* differ from the 129B6 F_1-*dydy* largely only by these allelic genes at the dystrophy locus, making them good normal and carrier controls. (A word of caution might be inserted regarding the high degree of genetic heterogeneity in offspring of 129B6 F_1 carriers; such progeny are not desirable for experimental use.)

Uniform 129B6 F_1-*dy* dystrophics exhibit clear-cut expression of the dystrophy syndrome at 2 to 3 weeks, and show progression of the disease during their extensive life-span. We anticipate they will withstand shipping better than do 129/Re-*dydy* mice. Small numbers of 129B6 F_1-*dy* dystrophics have been used at the Roscoe B. Jackson Memorial Laboratory for histological studies at 2 and 6 weeks postnatal (*8*), determinations of glycine transamidinase activity (*9*), measurements of acetoacetate accumulation (*10*), and experiments in artificial insemination (*11*). In these investigations results were obtained completely comparable with those in which 129/Re-*dydy* dystrophics were used. For many experiments, these 129B6 F_1 hybrid animals should give results identical with those obtained with 129/Re-*dydy* dystrophics. For other experiments, it may be necessary to establish new base lines fitting with the new background genetics. For some investigators the improvement in health and viability may not be worth this effort. However, we would like to suggest that these 129B6 F_1-*dy* dystrophics and their normal counterparts may become the animals of choice for many types of research (*12*).

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Inactivity in vivo of Transcortin-Bound Cortisol

Abstract. By means of a liver glycogen maintenance assay for corticosteroids, it was shown that transcortin-bound cortisol is biologically inactive.

From indirect evidence we previously postulated that transcortin-bound cortisol appears to be biologically inactive (*1*). Now we report experimental verification of this hypothesis, based on the observation that mice treated with transcortin were unable to respond to cortisol in a standard glycogen maintenance assay (*2*).

Transcortin was isolated from the plasma of patients with carcinoma of the prostate, who had been on diethylstilbesterol therapy (15 mg/day) for over 3 months. This type of plasma was chosen because of its high concentration of transcortin (*3*). Similar concentrations prevail during the third trimester of pregnancy (*1*). Although there is a possibility that the transcortin in the plasma of the cancer patients may differ from that in the plasma of normal or pregnant subjects, data obtained to date indicate that there is no difference in the physiological action of these transcortins (*1, 3, 4*). Furthermore, the chemical and physical properties of transcortin isolated from normal plasma are similar to those of the transcortin isolated from the serum of estrogen-treated patients with cancer of the prostate (*5*). The method of isolation, details of which will be published elsewhere, consisted of a gradient from 0.05*M* to 0.075*M* sodium chloride at a constant *p*H of 5.0 on a column of diethylaminoethylcellulose. The material eluted just prior to caeruloplasmin was used for injection.

Cortisol was assayed in adrenalectomized male Swiss mice according to published directions (*2*), with the exception that instead of adrenal cortical extract the animals received subcutaneously 100 μg of cortisol in 0.25 ml of corn oil after adrenalectomy and 200 μg of cortisol in 0.4 ml of corn oil 16 hours before commencement of the assay. At zero time each mouse received intravenously an amount of transcortin, which was calculated from binding data for each batch of transcortin to bind a minimum of 80 to 90 percent of the administered cortisol. Each animal then received 10 or 15 μg of cortisol subcutaneously in seven divided doses at hourly intervals. In one control group the transcortin was omitted, in the other the cortisol. Liver glycogen was assayed by the anthrone method (*6*), care being taken to add the anthrone reagent to the tubes while they were immersed in an ice bath. After the tubes had been heated for 10 minutes in boiling water, they were returned to the ice bath. This modification gave greater uniformity. The optical density of the greenish solutions was read in a Beckman model B spectrophotometer at 630 mμ.

From the results given in Table 1 it is evident that transcortin by itself has no demonstrable effect on the levels of liver glycogen. On the other hand, 10 to 15 μg of cortisol produce a definite response. The levels of 6.2 and 7.1 mg of glycogen per 10 g body

Table 1. The effect of injected transcortin and/or cortisol on the deposition of glycogen in the livers of fasting adrenalectomized mice (T, transcortin; F, cortisol; S.E.M., standard error of the mean).

Amount injected		Expt. No.	Mice (No.)	Mean glycogen ± S.E.M. (mg/10 g of body wt.)
T (mg)	F (μg)			
0*	0*	1	5	1.7 ± 1.1
25	0	1	6	1.7 ± 0.7
25	10	1	4	1.0 ± 0.6
25	10	2	4	1.8 ± 1.0
5.6	15	3	6	1.1 ± 0.4
0	10	1	7	6.2 ± 1.6†
0	10	2	9	20.5 ± 2.3‡
0	15	3	7	7.1 ± 1.2§

* Corn oil only injected. † $P = .05$ (This and the following values give the probability that the group receiving only cortisol is not different from the group receiving transcortin plus cortisol. ‡ $P < .01$. § $P < .01$.

weight are in good agreement with those of Eggleston *et al.* for cortisone (*2*). The reason for the increased response in the second experiment is not known. Injection of transcortin at the start of the assay completely cancelled the effect of the cortisol. There was no significant difference between the values for transcortin and those for transcortin plus cortisol. Cortisol by itself, however, yielded glycogen values which were consistently and significantly higher than those for the combination of cortisol and transcortin.

These results support our hypothesis (*1*) that the biologically effective level of cortisol in the body is not related to the total plasma concentration of the steroid, but to that which is not bound to transcortin. Some further experiments should be aimed at showing that as the cortisol-to-transcortin ratio increases, the biological efficiency of the cortisol increases. The present limited availability of transcortin precludes such in vivo tests (*7*).

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Autoradiographic Resolution of Doubly Labeled Compounds

The simultaneous incorporation of two different radionuclides in a biological system may often be utilized to increase the sensitivity of experiments. Distinction between the nuclides can be made either by chemical separation of the labeled compounds or by a detection system based upon the physical characteristics of the nuclides involved (differences in half-life, energy, and nature of the decay emission). Many of these methods are time-consuming or require extensive instrumentation. Autoradiography can be used to overcome these objections to some extent.

Ficq reported the distinction between tritium and C^{14} by the use of thick emulsions (*1*), since there is a large difference in the energy of the emitted electrons (0.017 Mev and 0.15 Mev). It would be advantageous to use a similar simple method to distinguish P^{32} and C^{14}, which are widely used in biochemical studies. X-ray film placed against paper chromatograms containing these isotopes has been used to great advantage (*2*). In the case of C^{14} and S^{35}, the emitted electrons are totally absorbed by the film (Kodak "No Screen" x-ray film, with emulsion on both sides). When counting particular radioactive spots with a Geiger tube, pieces of film placed over other areas of the chromatogram completely shield the tube from other C^{14} and S^{35} sources (*2*). The range of the P^{32} electron (1.7 Mev) is so great as to preclude any such simple shielding. Therefore, it is possible to overlay a chromatogram with two sheets of standard x-ray film; C^{14} or S^{35} will darken only the closest film, while radiophosphorus exposes both sheets.

We have utilized this technique on chromatograms containing adenosine diphosphate (ADP)-P^{32} (kindly given to us by M. Singer) and ADP-8-C^{14} (obtained from S. Mudd). These compounds were spotted on Whatman No. 1 chromatography paper with 500 to 3000 count/min per spot (measured by a 1.4 mg/cm² end-window Geiger tube). In addition, an unlabeled mixture of adenosine mono-, di-, and triphosphates (AMP-ADP-ATP) was spotted on position 1 and detected by ultraviolet light. One-dimensional ascending chromatograms were run in 5-percent Na_2HPO_4 saturated with isoamyl alcohol (*3*). The dried chromatograms were titled with radioactive ink and placed under two sheets of film for 3.5 days. The developed films are shown in Fig. 1. These may be scanned with a recording densitometer as shown by the chart record. The ordinate is on a logarithmic scale, giving the optical density directly.

Adenosine diphosphate-8-C^{14} on position 2 and the radiocarbon title gave an intense exposure only on film 1, while ADP-P^{32} on position 3 darkened both films, the second film being twice as dark as the first. Chromatogram spots containing only C^{14} (or S^{35}) or only P^{32} are clearly distinguished.

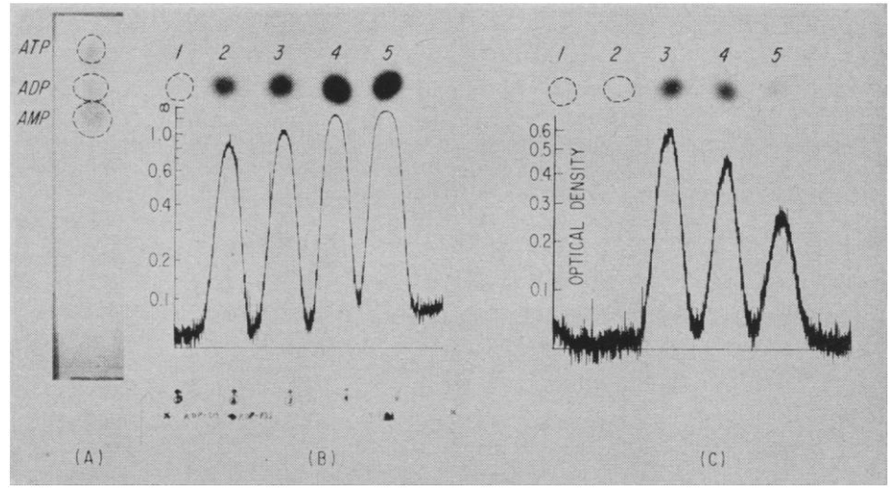

Fig. 1. Autoradiography of chromatogram containing ADP-C^{14} and ADP-P^{32}. Chromatogram was overlayed with two sheets of Kodak "No Screen" x-ray film. (*A*) Ultraviolet scan of AMP-ADP-ATP mixture; (*B*) and (*C*), the first and second films respectively. The title at the bottom of the first film (*B*) is a radiographic exposure from ink containing C^{14}. On original chromatogram, position 1 contained unlabeled nucleotide mixture. Spot 2 had 1445 count/min C^{14}. Spot 3 had 945 count/min P^{32}. Spot 4 had 1265 count/min C^{14} and 974 count/min P^{32}. Spot 5 had 2500 count/min C^{14} and 457 count/min P^{32}. Densitometer tracings of developed x-ray films are given below the spots.