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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20 November 1961

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.

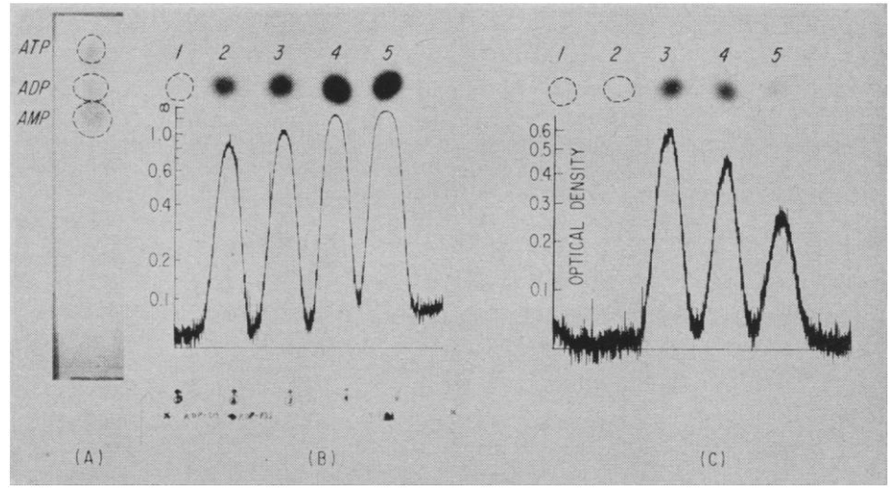

Fig. 1. Autoradiography of chromatogram containing ADP-C^{14} and ADP-P^{32}. Chromatogram was overlayed with two sheets of Kodak "No Screen" x-ray film. (*A*) Ultraviolet scan of AMP-ADP-ATP mixture; (*B*) and (*C*), the first and second films respectively. The title at the bottom of the first film (*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.

Compounds containing both radiophosphorus and radiocarbon are also detected. A mixture of C^{14} and P^{32} labeled ADP on spots 4 and 5 exposed both films with different intensities. If there is an equal quantity (in counts per minute) of the two nuclides in a given spot, the closest film will be about four times as dark as the second film. A large excess of C^{14} will expose the closest film proportionally more. The reverse situation, where a doubly labeled spot contains many-fold more P^{32} counts per minute than C^{14}, cannot be immediately resolved. However, the relatively short half-life of P^{32} (14.3 days) permits the resolution to be made after an appropriate decay. This would be more difficult in the case of P^{32}-S^{35} labeled compounds.

In general, when C^{14}-P^{32} or S^{35}-P^{32} chromatograms contain only one radionuclide per spot, visual comparison of the two films permits a simple identification of the material. Under some conditions, doubly labeled spots may also be resolved.

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16 October 1961

Strontium-90 in the 1959 United States Wheat Crop

Abstract. A broad sampling of the 1959 United States wheat crop has been analyzed for the presence of strontium-90. The level of strontium-90 found in these samples is in general agreement with the findings of other investigators, with the exception that the highest levels of strontium-90 in wheat were found in samples from Oklahoma.

An extensive investigation of the 1959 United States wheat crop for the presence of strontium-90 has been completed by this laboratory. Eighty-eight samples from 18 states were collected by nonscientific personnel at grain elevators and grain terminals within the respective states. The samples were taken from wheat mixtures from crop areas surrounding the elevators and terminals. All samples were transmitted to this laboratory for analysis.

Table 1. Strontium-90 content of wheat samples by states.

State	Samples (No.)	Picocuries/kilogram			Picocuries/gram of calcium		
		Average	Max.	Min.	Average	Max.	Min.
Arizona	1	9.4			24.4		
California	4	7.6	14.7	4.7	22.7	52.6	11.9
Colorado	4	25.0	43.6	10.1	51.9	104	19.4
Idaho	8	19.4	48.5	9.9	41.4	78.7	22.0
Illinois	3	55.4	69.1	25.5	174	231	75.2
Indiana	9	53.0	85.7	25.6	135	232	67.4
Kansas	8	50.9	93.4	24.0	119	222	47.0
Minnesota	4	38.7	46.7	30.8	117	154	88.2
Missouri	3	44.5	54.0	36.7	134	164	105
Montana	8	29.9	40.3	17.7	85.4	104	51.5
Nebraska	5	54.9	80.0	36.7	141	211	96.5
Nevada	1	7.7			17.6		
North Dakota	8	27.4	44.3	18.7	95.7	158	62.2
Ohio	5	51.3	68.6	29.1	140	196	76.8
Oklahoma	8	82.8	132	59.9	233	366	182
South Dakota	3	30.1	35.6	23.1	79.2	85.5	68.5
Texas	5	58.6	85.1	44.3	125	181	92.4
Utah	1	19.2			38.4		

The preparation of wheat samples for analysis was straightforward. Seventy-five grams of wheat were ground in a pin mill and then ashed at 450°C for 20 to 24 hours. The inorganic residue was dissolved with a small amount of concentrated nitric acid. Strontium nitrate carrier was added, and the sample was digested 1 hour on a steam bath to promote isotopic exchange. Essentially all of the ash was dissolved by this procedure.

The amount of strontium in the wheat was determined by the fuming nitric acid method of Martell (*1*). The dissolved sample was treated with fuming nitric acid, and strontium was precipitated as the nitrate. After two nitrate precipitations, the precipitate was dissolved in water, and yttrium was precipitated as a hydroxide. The yttrium-free Sr^{90} was held for 10 to 14 days to permit the Y^{90} daughter to accumulate to radioactive equilibrium. The yttrium was again separated as a hydroxide and then precipitated for counting as an oxalate. Strontium was precipitated and counted as a carbonate. The counting was done in a Tracerlab CE-14 low-background counter which had been standardized with K^{40}, Sr^{90}, and Y^{90}.

The calcium content of the wheat was determined in a separate analysis. The results of the calcium analysis were used in computing picocuries of Sr^{90} per gram of calcium.

One wheat sample was analyzed by an independent laboratory, Nuclear Science and Engineering Corporation. The same sample was also analyzed by the Minnesota Department of Health. The results of the three laboratories were in agreement.

Table 1 shows the results of our analyses (*2*). Strontium-90 levels varied widely between samples within any particular state. When duplicate runs were made, however, the duplicate results were in agreement with the initial runs. The results are listed both in picocuries per kilogram of wheat and picocuries per gram of calcium. The highest levels were found in the south central part of the United States, with very low levels in the western United States.

The results of this survey show levels in the upper Midwest to be slightly lower than those reported by Caldecott for the 1958 wheat crop (*3*). On the other hand, the findings for Texas and Oklahoma wheat are somewhat higher. It is possible that these differences may be due to rainfall variations in the sampled areas from one year to the next.

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5 October 1961