and 0.5 ml of 0.2 *N* tetraethyl ammonium hydroxide. Nitrogen was bubbled through the solution for 15–20 min and the polarogram recorded. In this investigation, as in the study of the alcoholic solutions of digitoxin, multiple runs were made until a satisfactory height-versus-concentration curve was obtained and the average half-wave potential was calculated.

The results of these experiments show that digitoxin may be determined in concentrations as low as 0.1 μg in both alcoholic solutions and in blood. Multiple determinations run on concentrations between 0.1 and 0.4 μg of digitoxin in blood show the error in this method to be ±0.02 μg. Fig. 1 shows the height of polarographic break for various concentrations of digitoxin in alcoholic solutions and in blood extracts. This figure also shows the relationship between height of break for alcoholic and blood determinations. It may be seen that in concentrations down to approximately 0.6 μg the two curves coincide reasonably well; however, below this concentration the curve of digitoxin extracted from blood drops sharply and approaches zero. This drop from the alcoholic curve may be due to the distribution of digitoxin between the extraction solvent and blood at these low concentrations. The use of the arbitrary curve, although it varies from the curve of digitoxin in alcoholic solution, is based upon the results of multiple determinations which show a low error (± 0.02 μg) in the concentrations where the deviation is greatest. The average half-wave potentials were found to be −1.965 in alcoholic solution and −1.958 when extracted from blood.

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Effect of Sulfadiazine on Survival of the Mammalian Embryo[1]

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It has been shown by Detwiler, Copenhaver, and Robinson (*2*) that sulfadiazine in solutions of 1% or higher is frequently toxic to *Amblystoma* embryos in early developmental stages. More recently, Copenhaver and Detwiler (*1*) showed that 2% concentration of sulfadiazine caused a failure to survive to the stage of yolk resorption. They also observed abnormalities of various structures in the organism. S. Y. P'an (*4*) has shown that sulfamethiazine when administered to normal male rats produces gross and microscopic atrophic changes in the testes, seminal vesicles, and anterior prostate. Recently, Figge *et al.* (*3*) reported on the influence of sulfonamide drugs on cancer susceptibility, and reproduction in mice. He has observed that there is a decrease in reproduction in mice maintained on constant medication with certain sulfonamides. Two of the authors (Yntema and Hammond) have found that sulfadiazine is more lethal to chick embryos during the first half of the second day of incubation than it is subsequently. The drug appeared to interfere with the development of the vascular system. These studies suggested that sulfonamide administration might interfere with early development of the mammalian embryo.

TABLE 1

Expt. No.	Treatment*	Given on days	No. of mice	No. of deliveries (full term)	Percentage deliveries	χ^2	P
1	Untreated		56	27	48.2	7.64	0.0057
	Sulfadiazine†	5–15	56	13	23.2		
2	Untreated (isocaloric)		49	16	32.7	1.6	0.109
	Sulfadiazine	5–15	47	10	21.3		
3	Sulfadiazine	8–12	79	33	41.8	15.24	0.0001
	"	6–10	79	11	13.9	10.84	0.001
	"	10–14	79	29	36.7		
4	Sulfadiazine	6	109	15	13.8	23.18‡	
	"	7	104	15	14.4	22.39‡	

* Administered only in the diet in Experiments 1–3. In Experiment 4, one injection of 12 mg sulfadiazine sodium was given intraperitoneally, in addition to administration of sulfadiazine in the diet for the 6th or 7th day.

† Eight-tenths percent of diet.

‡ Compared with untreated controls in Experiment 1.

To investigate this, we selected white mice as our experimental animal and used sulfadiazine as a representative of the sulfa group of drugs. A diet of Purina dog chow containing 0.8% by weight of sulfadiazine was employed. The breeding cages contained four females and one male. Females were examined each morning. Those having vaginal plugs were removed, numbered, and placed in individual cages. Only the females showing vaginal plugs were selected for the investigation.

Experiment 1 (Table 1) represents the results obtained when such animals, beginning on the 5th day following conception, were fed sulfadiazine in the diet for a period of 10 days. The percentage of deliveries in this treated group was 23.2%, in contrast with 48.2% in the untreated control group. When untreated animals were fed isocalorically there was a reduction of deliveries, as indicated by the results of Experiment 2. However, this reduction did not reach the level noted in the sulfadiazine-treated animals.

It was of interest to localize more accurately the time at which the sulfonamide was effective. Experiment 3 indicates the results. Here it was shown that the period from the 6th to the 8th day was the one at which the sulfonamide was effective.

To localize further the most effective interval, Experiment 4 was conducted, in which animals were given sulfonamide for 1 day only. Here sulfadiazine sodium was

[1] This investigation was supported by a research grant from the Hendricks Fund at Syracuse University.

administered intraperitoneally, so that initial high sulfadiazine levels could be obtained. Following the intraperitoneal injection, the mice were placed on the sulfadiazine diet for 24 hr. Similar results were obtained whether sulfonamide was administered only on the 6th or only on the 7th day following appearance of the vaginal plug. No data have been obtained concerning the period from the 1st to 6th day.

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Enzymatic Reduction of 2,4-Diketo Acids Catalyzed by Dihydrodiphosphopyridine Nucleotide

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The reduction of pyruvic acid to lactic acid, catalyzed by dihydrodiphosphopyridine nucleotide ($DPNH_2$) in muscle extracts is well known. An apparently analogous reaction in which 2,4-diketo acids are reduced has been observed. When a series of homologous 2,4-diketo acids was incubated with 90% pure $DPNH_2$[1] and an aqueous extract of an acetone powder of rabbit muscle at pH 7.2, the characteristic absorption band of these compounds at 2900 A and the band due to $DPNH_2$ at 3400 A disappeared progressively. Equimolar amounts of coenzyme and diketo acid were utilized, suggesting the reduction of one keto group. In a typical experiment using a system containing 5×10^{-7} moles each of 2,4-diketovalerate and $DPNH_2$, 10^{-4} moles of phosphate buffer at pH 7.2, and 80 γ of protein nitrogen per 3 ml, 2.48×10^{-7} and 2.44×10^{-7} moles of $DPNH_2$ and diketo acid, respectively, disappeared after 12 min of incubation at 25° C. Neither $DPNH_2$ nor substrate disappeared when one of these was omitted from the system or in the absence of enzyme. No lactate was formed as determined by the method of Barker and Summerson (*1*), ruling out prior hydrolysis of the diketo acid to pyruvic acid (*3*). The facts are compatible with the equation,

$$RCCH_2CCOOH + DPNH_2 \rightarrow RCCH_2CHCOOH + DPN$$
$$\quad\;\; \| \quad \| \qquad\qquad\qquad\qquad\quad \| \quad |$$
$$\quad\;\; O \quad O \qquad\qquad\qquad\qquad\quad O \quad OH$$

whereby the product is considered tentatively to be the 2-hydroxy-4-keto acid.

The reaction proceeded more rapidly with increasing concentrations of substrate and was conveniently followed spectrophotometrically by measuring the rate of decrease of the $DPNH_2$ band at 3400 A. All of the normal 2,4-diketo acids from valeric to undecylic were reduced in the system, as shown in Table 1. Under the same experimental conditions, neither 4-keto valeric acid nor 3,5-diketohexanoic acid was reduced. The nature of the enzyme involved and its possible relationship to lactic dehydrogenase is under investigation.

TABLE 1

Enzymatic Reduction of 2,4-Diketo Acids*

2,4-Diketo acid	Disappearance of $DPNH_2$
	(Moles $\times 10^{-7}$ per min)
n-Valeric	1.83
n-Hexanoic	1.74
n-Heptanoic	1.45
n-Octanoic	1.48
n-Nonanoic	1.36
n-Capric	1.33
n-Undecylic	1.45

* Composition of system in moles per 3 ml was 5×10^{-7} $DPNH_2$, 9×10^{-6} diketo acid, 1×10^{-4} phosphate buffer (pH 7.2); and 0.1 ml enzyme preparation (100 γ protein nitrogen) per 3 ml; 25° C.

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A Convenient Quick Method of Obtaining Vitamin B_{12} Concentrate[1]

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The nonprotein filtrate of liver homogenate (proteins coagulated by boiling at pH 5.0) chromatographed on starch columns by the method of Moore and Stein (*1*) gave a reddish brown fraction in the first portions of the effluent.

The behavior and color of this fraction suggested a possible relation to B_{12}. This was tested as follows: 0.5 ml of liver injection, USP (Lederle Solution Extract, from beef liver, 15 u per ml) was dried by blowing air across it at room temperature. To the residue 0.1 ml 1*N* HCl was added, and then 0.5 ml of a mixture consisting of 0.1 *N* HCl, *n*-propanol, and *n*-butanol in the proportions 1: 2: 1. The solution was chromatographed on 25 g starch in a 10-mm × 300-mm column with the 1: 2: 1 mixture as solvent.

[1] This work is part of that done under a joint contract with the Office of Naval Research, United States Navy Department, and the United States Atomic Energy Commission.

[1] DPN was purified by countercurrent distribution as described by Hogeboom and Barry (*2*). Cruder preparations tended to interfere with measurements made at 2900 A.
m = calibration factor for adrenalin