TABLE I.
Influence of Vitamin B_{12} and Methionine on Incidence of Hepatomas in p-Dimethylaminoazobenzene-Fed Rats.

Supplement to diet	Avg daily food intake, g	Avg daily wt gain, g	Incidence of hepatomas, %	Liver wt*— % of body wt
None	6.4	0.1	17	9.3
Vitamin B_{12}	7.0	0.2	78	15.7
Methionine	8.1	1.2	11	7.0
Methionine + vit. B_{12}	10.5	1.2	33	11.0

* Liver weights include tumors, where present.

diet supplemented with both vitamin B_{12} and methionine. The rats were fed these diets for 170 days and were then killed and autopsied. The incidence of gross hepatomas was recorded. There were 9 rats in each group. Three rats in the unsupplemented group died without hepatomas during the experimental period. Two rats receiving vitamin B_{12}, one rat receiving methionine, and one rat receiving vitamin B_{12} plus methionine died with hepatomas during the experimental period. The incidence of hepatomas is based on those rats either surviving the 170-day period or dying with hepatomas.

Results and discussion. The pertinent data are presented in Table I. The addition of vitamin B_{12} to the DAB-containing, methionine-deficient diet increased the incidence of hepatomas from 17% to 78%. The addition of vitamin B_{12} to the methionine-containing diet increased the incidence of hepatomas only from 11% to 33%; thus methionine appeared to afford some protection.

The procarcinogenic effect of vitamin B_{12} may indicate that this vitamin is required for tumor growth or it may be a reflection of an influence of vitamin B_{12} on metabolic transformations of the carcinogen p-dimethylaminoazobenzene. In the former event these results might have general application to the entire field of cancer. Experiments are in progress to elucidate the mechanism of the procarcinogenic effect of vitamin B_{12}.

It would be unfortunate if the conclusion were drawn from these experiments that vitamin B_{12} itself is carcinogenic. There is no evidence that such is the case. On the contrary, a control group of rats receiving the soybean protein diet with vitamin B_{12}, but without p-dimethylaminoazobenzene, showed no hepatic tumors.

Summary. Vitamin B_{12} has been found to enhance markedly the carcinogenic effect of p-dimethylaminoazobenzene in rats receiving a methionine-deficient diet. However, a control group of rats receiving this diet without p-dimethylaminoazobenzene showed no hepatic tumors.

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Life Maintaining Activity of Δ^1 Desoxycorticosterone Acetate.* (18068)

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Life maintaining activity in adrenalectomized rats has been obtained with Δ^1 allopregnen-21-ol-3, 20-dione 21 acetate for which the trivial name Δ^1 desoxycorticosterone acetate was suggested(1). This steroid has been compared with desoxycorticosterone acetate (Percorten, Ciba) for life maintaining

* This investigation was done under contract with the Office of Naval Research, Navy Department.

1. Djerassi, C., Scholz, C. R., and Leathem, J. H., *Experientia*, 1949, v5, 204.

TABLE I.
Survival of Adrenalectomized Immature Male Rats Treated with Desoxycorticosterone Acetate or Δ^1 Desoxycorticosterone Acetate.

Daily treatment mg	No. of rats, ADX	No. rats surviving at 10 days	15 days	20 days	Avg body wt increase in 20 days, g
		Desoxycorticosterone acetate			
0.025	10	7	4	4	+20
0.050	10	10	10	9	+48
0.100	10	10	10	10	+69
		Δ^1 Desoxycorticosterone acetate			
0.10	9	7	2	0	—
0.25	11	11	11	7	+23
0.50	10	10	8	8	+45
None	8	0			—

and body weight increasing activity.†

Male rats of the Long-Evans strain were used when approximately 60 g in body weight. The rats were bilaterally adrenalectomized and fed *ad libitum* a diet consisting of 18% casein (Labco), 60% dextrose, 10% yeast, 5% Wesson's salt mixture, 5% Mazola and 2% cod liver oil. The steroids were dissolved in peanut oil and injected subcutaneously once daily.

Ten normal male rats gained an average of 69 g body weight in 20 days whereas untreated adrenalectomized rats lost weight and died 4 to 8 days after the operation. Daily injections of peanut oil alone (0.2 cc) did not alter survival time. The pertinent data obtained with the steroids are presented in Table I and indicate that Δ^1 DCA is about 1/8 to 1/10 as potent as DCA. 0.25 mg and 0.50 mg of Δ^1 DCA daily permitted good survival and approximately 1 and 2 g gain in body weight daily respectively. Normal body weight increases and 100% survival was obtained with 0.1 mg DCA daily. Thayer (2) has previously shown that 0.1 mg of DCA is necessary to permit normal growth and complete survival in immature adrenalectomized rats. For 80% survival and 1 g increase in body weight daily, dosages of 20 μg(2) and 67 μg(3) of DCA have been reported. Our data with DCA indicate that an 80% survival is associated with a 2 g daily body weight gain.

Cessation of Δ^1 DCA administration after 20 days of 0.5 mg daily resulted in death 6 and 9 days later with 2 rats. Discontinuing DCA after 20 injections of 0.025 mg resulted in death in 3 rats after 3 days whereas after 0.05 mg daily, 6 rats died 5, 6, 8, 9, and 10 days after the last injection.

The livers from normal and adrenalectomized rats maintained for 20 days on both steroids were dried to constant weight at 95°C to determine water content. Groups of 4 to 7 rats were studied and liver water content was normal in all cases. The dried livers from two groups of adrenalectomized rats on Δ^1 DCA for 20 days and from normal rats were analyzed for total nitrogen and the data converted to protein by the factor 6.25. Adrenalectomized rats maintained on 0.25 mg and 0.50 mg of Δ^1 DCA had liver protein values of 0.85 g and 0.87 g/100 g body weight respectively. The livers from normal rats contained an average 0.84 g protein/100 g body weight and indicated that relative liver protein content was the same in both groups.

To observe any organ weight changes which Δ^1 DCA might induce, 0.5 mg was injected subcutaneously for 10 days into five 22-day-old female rats and the organ weights compared with an equal number of littermate controls. Weights of the hypophysis, adrenal, thyroid, ovary, uterus, thymus, heart, spleen and kidney were unchanged. Liver weight

† The steroids were generously supplied by Ciba Pharmaceutical Products, Summit, N. J.

2. Thayer, S. A., *Vitamins and Hormones*, 1946, v4, 311.

3. Kuizenga, M. H., Nelson, J. W., and Cartland, G. F., *Am. J. Physiol.*, 1940, v130, 298.

was somewhat greater in the injected rats being 5.3 g/100 g body weight as compared with 4.6 g/100 g body weight in controls.

Summary. The steroid Δ^1 desoxycorticosterone acetate will maintain adrenalectomized immature rats for 20 days with dosages of 0.25 mg and 0.5 mg and will increase daily body weight 1 and 2 g respectively. Activity is approximately 1/8 to 1/10 that of desoxycorticosterone acetate. Liver water content was unchanged in maintained adrenalectomized rats and the relative liver protein content of adrenalectomized rats receiving Δ^1 desoxycorticosterone acetate was comparable to that of normal rats. This steroid did not alter organ weights in normal immature female rats over a 10-day period except for a tendency toward liver weight increase.

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Growth Requirements of *Streptococcus mitis* and Sulfonamide Resistance.* (18069)

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Previous work in our laboratory showed that trained sulfathiazole-resistant strains of *Streptococcus mitis* differed considerably in other biochemical properties from the original strains(1,2). The resistant strains acquired the ability to grow on 40% bile agar, in 6.5% NaCl, in .1% M.B. milk, and in the presence of increased concentrations of 4 antibiotics. These properties are characteristic of enterococci(3), and were shown by a known strain of *Streptococcus fecalis*, No. 9790, obtained from the American Type Culture Collection.

The growth requirements of an organism are fundamental properties sometimes suggesting a possible mechanism for their response to bacteriostatic agents. Since we had observed that the resistant strains grew readily in a medium containing no riboflavin (Bacto Riboflavin Assay Medium), while the parent strains did not, we have investigated the vitamin requirements of several parent and resistant strains of *S. mitis* and of *S. fecalis*, No. 9790. This paper reports the results of that study.

Experimental. Semi-synthetic medium. This was a modification of the medium used by Smiley, Niven, and Sherman in their study of the growth requirements of *S. salivarius*(4). It was prepared in two stages, Part A being autoclaved prior to addition of Part B. Each batch of medium, upon the addition of the vitamins, was filtered through a Seitz filter.

Organisms used. Five strains of streptococci showing the biochemical characteristics of *Streptococcus mitis*, isolated in our labora-

PART A

Casein hydrolysate, 10% solution*	50 ml
DL-Tryptophane	60 mg
Dipotassium phosphate	6.0 g
Glucose	5.0 g
Sodium thioglycollate	100 mg
" chloride	2.0 g
Magnesium sulfate, cryst.	80 mg
Ferrous sulfate, cryst.	4 mg
Manganese chloride	1.2 mg
Uracil	10 mg
Water to	1,000 ml
pH 7.5	

* Vitamin-free casein hydrolysate (acid) 10% solution prepared by Nutritional Biochemicals Corp., Cleveland, Ohio.

* A portion of the data presented in this paper was taken from a thesis submitted in June, 1950, by Virginia M. Harrison to the University of Colorado in partial fulfillment of the requirements for the degree Master of Science.

1. Clapper, W. E., and Heatherman, M. E., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 392.

2. Clapper, W. E., and Heatherman, M. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 153.

3. Swift, H. F., *The Streptococci. Bacterial and Mycotic Infections of Man*, J. B. Lippincott Company, Philadelphia, 1948.

4. Smiley, K. L., Niven, C. F., and Sherman, J. M., *J. Bact.*, 1943, v45, 445.