The growth substances tested in these experiments were indole-3-acetic acid (I-3-E) and an extract of Brussels sprouts; the inhibitors were eosine and a *Syringa* extract. Values for Z in the mixture I-3-E/eosine in different concentrations are shown in Fig. 1. The curves of the other possible mixtures of the above-mentioned substances were analogous but differed from those of Fig. 1 because of varying efficacy of the original substances.

The following ideas about the mode of action of the growth substances and growth inhibitors provide a possible explanation of the results.

The growth-inhibiting or growth-promoting substances must be converted into a part of the living substance, being adsorbed by molecules of "living structure." It is reasonable to postulate that in the molecular system of the living substance there are spaces that can be occupied by the molecules of the growth regulator. This means that the molecule of the growth regulator has a certain affinity for a certain "space." In this connection the ideas of R. Kuhn (*4*) about the mode of action of the sulfonamides may be recalled.

Our hypothesis postulates two kinds of spaces: The first filled in by growth substances effects a growth promotion; the second type also shows a certain affinity, but in this case growth inhibition results.

It is immaterial whether there is a molecular process of self-reproduction, as postulated by Dehlinger (*5*), Neugebauer (*6*), Jordan (*7*), or Friedrich-Freska (*8*), with the growth substance as a "brick" of the molecule of the living substance, or whether the active substance is effective by adsorption in a certain place with a specific metabolic function influencing the growth process.

Any organic molecules brought from outside into the plant will not ordinarily be able to fill the "spaces" of the growth substances with the necessary active compound for the growth effect. The molecules accumulate somewhere on the living structures, where they disturb their functions and exert an inhibiting action. The growth substances, besides promoting growth, have also an inhibiting effect, although the latter is less probable. Thus every growth substance consists of a promoting and an inhibiting compound.

From these theories, the following equation, which should apply to the experimental results, and which shows the relation between the increase of elongation (Z) and concentration of growth substances (c), can be derived:

$$Z = A(1 - e^{-k_1 c_1}) - B(1 - e^{-k'_1 c_1}) .$$

For growth inhibitors the formula is:

$$Z = -C(1 - e^{-k_2 \sqrt{c_2}}).$$

Finally, for mixtures of growth substances and growth inhibitors, the formula is:

$$Z = A(1 - e^{-k_1 c_1 e^{-l_2 \sqrt{c_2}}}) - B(1 - e^{-k'_1 c_1 e^{-l'_2 \sqrt{c_2}}}) - C(1 - e^{-k_2 \sqrt{c_2} \cdot e^{-l_1 c_1}}).$$

A, B, and C denote figures, which are proportional to the number of places of adsorption ("spaces") mentioned above; k and l denote probabilities of hits per unit of concentration. If the constants are chosen correctly, the theoretical curves can be brought into agreement with the curves established by the experiments. The curves for the mixtures I-3-eosine (Fig. 1) could be derived mathematically from the values for the different substances with good conformity.

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Desoxyribonucleic Acid Content of Rat Liver Nuclei Influenced by Diet

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That the desoxyribonucleic acid (DNA) content of all diploid cells of an animal, and the species, is constant has been suggested by a number of investigators (*1–4*). This would exclude the possibility that diet influences the DNA content of nuclei. Kosterlitz *et al.* (*5, 6*) and Davidson (*7*) suggested that the DNA content of liver nuclei of rats was not influenced by fasting or by protein-free diets.

Nuclei in sections of livers of rats on a protein-free diet were observed to stain more intensely by the Feulgen nuclear reaction than those of rats on the laboratory stock diet, and the results of the present investigation obtained by both chemical and cytochemical methods indicate that the diet does influence DNA content of nuclei of rat livers.

Male rats from the Albino Farms, Red Bank, N. J., 130–160 g, were maintained on one of three diets: Fox Food Blox (Allied Mills), which contained a minimum of 26% protein; a semisynthetic diet containing 12% washed casein (Eimer and Amend); and a protein-free diet (Table 1).

Nuclei were prepared from the livers with 5% cold

TABLE 1

COMPOSITION OF DIETS

	12% casein diet (%)	Protein-free diet (%)
Casein	12.0	0.0
Corn oil	5.0	5.0
Dextrose	77.0	89.0
Salt mixture	4.0	4.0
Rice bran extract (Vitab)	2.0	2.0
Riboflavin	0.002	0.002

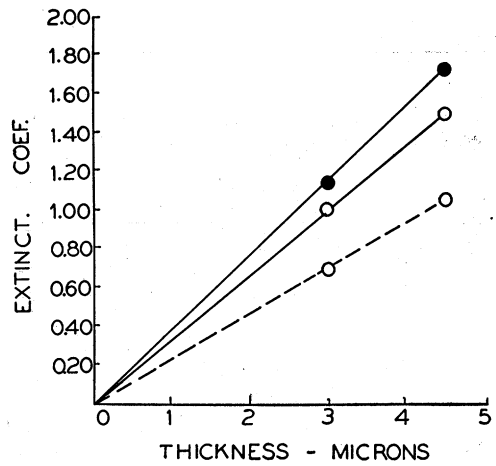

FIG. 1. Relation of density of Feulgen color in nuclei to thickness of sections. ●——●, Protein-free diet; ○——○, 12% casein diet; ○----○, Fox Food Blox diet.

citric acid by the method described by Marshak slightly modified (*8*) after the livers had been perfused *in situ* with cold 0.85% sodium chloride solution.

The number of nuclei per ml of the nuclear suspensions was determined by means of a blood cell counting chamber. The amount of DNA in known numbers of nuclei was determined by a slight modification of the method described by Kosterlitz (*5*). The suspensions contained all types of liver nuclei. A comparison of the relative amount of DNA in the hepatic nuclei was made by a photometric comparison of the Feulgen color in the nuclei of sections. Tissue specimens from each group were mounted together and cut with the same stroke of the microtome knife; this assured equal thickness of sections. Ten oil immersion fields per section were photographed with green light (Wratten B Filter, 4-mm obj., 10 × ocular) and constant exposure times.

Densitometric measurements were made as described elsewhere (*9*). The circular, or slightly elliptical, film images of hepatic nuclei were projected through a circular opening onto the photoelectric element of the densitometer. The opening in the densitometer was great enough to admit the whole projected nuclear image. The fit of image into the opening was not always exact, and with slightly oval images some of the background field was admitted. This introduced a slight error common to all sections. Approximately 100 nuclei were measured from each liver. The accuracy of the method was tested by measurements of sections cut at 2 different thicknesses. Fig. 1 shows that as thickness of section was increased there was a corresponding increase in Feulgen color.

In order to determine if any differences found in the DNA content of the nuclei from the three dietary groups were due to changes in ploidy, the diameters of a minimum of 10,000 nuclei (from the nuclear suspension from 10 rats) of each group were measured with a filar micrometer. All spherical nuclei, regardless of size, were measured. The results of the measurements of nuclei of each group were condensed so that the intervals are 1 μ and extend from 4.5 to 18 μ. The volume of the average nucleus at each interval was calculated on the assumption that the nuclei were spherical.

It may be seen in Fig. 2 that the nuclei of the livers of the rats fed the protein-free diet were the smallest of the three groups, those of the 12% casein diet intermediate, and those of the Fox Food Blox the largest. The graph does not include a small number of the largest nuclei of the groups; there was no appreciable difference between the groups among these nuclei.

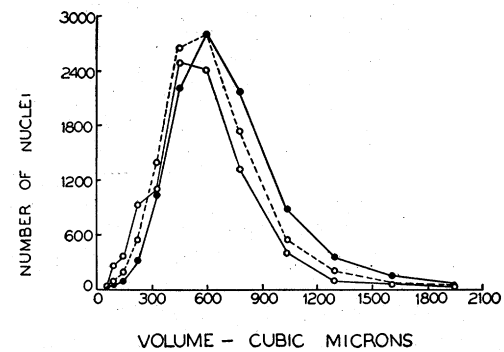

FIG. 2. Comparison of volumes of nuclei of livers from rats fed different diets. ○——○, Protein-free diet; ○----○, 12% casein diet; ●——●, Fox Food Blox diet.

The mean volumes of the nuclei from the three dietary groups were as follows: Fox Food Blox diet, 572 cu μ (10,196 nuclei); 12% casein diet, 494 cu μ (10,126 nuclei), or 13.6% smaller than those from the Fox Food Blox diet group; protein-free diet group, 372 cu μ (10,280 nuclei), or 35% smaller than those from the animals fed the Fox Food Blox diet.

The results of the chemical determinations of DNA in isolated liver nuclei and of the photometric comparison in histological sections of the amount in the hepatic nuclei of livers of rats of the three dietary groups are shown in Table 2, *a* and *b*. It may be seen that with the diet of Fox Food Blox the average nucleus, although largest in volume of the three groups, contained the smallest amount of DNA, 1.02×10^{-8} mg; with the 12% casein diet the nuclei contained an average of 1.17×10^{-8} mg DNA/nucleus; with the protein-free diet, in which group the nuclei were the smallest, the nuclei contained the most DNA per nucleus, 1.20×10^{-8} mg. The values were approximately 18% greater with the protein-free diet and 15% greater with the 12% casein diet than with the Fox Food Blox diet.

The DNA content of the hepatic nuclei, compared photometrically (Fig. 3), was 50% greater ($P < 0.01$) in the rats fed the protein-free diet than in those from the rats fed the Fox Food Blox diet, although the nuclei in the latter were approximately 14% larger than those of the other two groups, which

TABLE 2

DNA CONTENT OF RAT LIVER NUCLEI

	No. rats	Days on diet	Diet	DNA/nucleus (chemical method) mg	Relative amount of DNA in nuclei (cytochemical method) extinction coefficient	Relative size of nuclei†
	27	8–49	Fox Food Blox	$1.02\ (\pm 0.009^*) \times 10^{-8}$		
a	26	"	12% Casein	$1.17\ (\pm 0.010\ ) \times 10^{-8}$		
	27	"	Protein-free	$1.20\ (\pm 0.008\ ) \times 10^{-8}$		
	9	15–40	Fox Food Blox		0.723 ± 0.084	2.563 ± 0.486
b	9	"	12% Casein		0.961 ± 0.092	2.240 ± 0.094
	9	"	Protein-free		1.090 ± 0.063	2.241 ± 0.136
	5	60	Fox Food Blox	0.98×10^{-8}		
	5	30	12% Casein +	1.00×10^{-8}		
c		30	Fox Food Blox			
	5	30	Protein-free +	1.00×10^{-8}		
		30	Fox Food Blox			
	5	60	Fox Food Blox		0.808 ± 26.5	
	5	30	12% Casein +		0.808 ± 11.7	
d		30	Fox Food Blox			
	5	30	Protein-free +		0.844 ± 14.4	
		30	Fox Food Blox			

* Standard error of mean.

† Weights of 100 cutouts of projected nuclear images.

were equal in size (Table 2, *b*). The DNA content of the hepatic nuclei from the rats of the group fed the 12% casein diet was also greater (33%) than that of the Fox Food Blox diet fed group.

When groups of rats were fed the three diets for 30 days, and then fed Fox Food Blox diet for an additional 30 days, no significant difference ($P > 0.05$) was found in the DNA content of the nuclei of the livers from the three groups (Table 2, *c* and *d*).

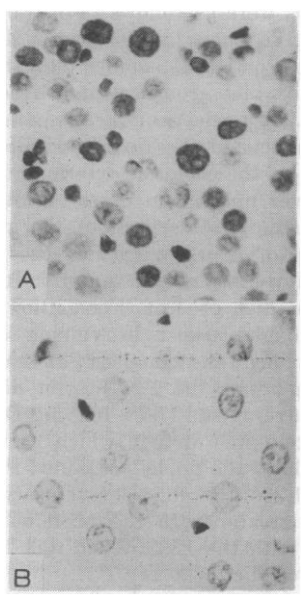

FIG. 3. Feulgen-stained sections of rat liver. (×450.) *A*, Protein-free diet; *B*, Fox Food Blox diet. The sections were mounted and stained on the same microscopic slide, photographed on the same strip of film with equal exposures; the prints were made under identical conditions of exposure and development. The intensity of Feulgen color varies considerably among the nuclei.

The average DNA content of 1.02×10^{-8} mg/nucleus for rat liver is in agreement with that found by Dounce and co-workers (*10*) and with that of Day (*11*), who reported an average of 1.02×10^{-8} mg/nucleus based on the thymine content.

The increase in nuclear DNA content caused by the diets apparently cannot be accounted for on the basis of increase in polyploidy if size of nuclei is used as the criterion of polyploidy, since the values for DNA were in the reverse order of nuclear size.

There appears to be a definite relationship between nuclear DNA content in the rat liver and the nutritional state of the animal. For example, three groups of 10 rats each, initially equal in weight, fed the three diets for 8–37 days, had average final weights of: Fox Food Blox diet, 278 g; 12% casein diet, 177 g; protein-free diet, 117 g. The DNA contents of the nuclei of the three groups were in the reverse order of magnitude. In the experiments the nuclear DNA content was increased when the protein content of the diet was decreased.

These results do not support the suggestions made by Kosterlitz (*5*), Campbell and Kosterlitz (*6*), and Davidson (*7*) that fasting and protein-free diets do not influence the DNA content of nuclei. A probable explanation for the difference between these results and those of Davidson is that in Davidson's experiments rats were fasted for 24 hr, whereas the experimental period in our experiments varied from 8 to 49 days. The evidence presented by the other investigators appears to be indirect; the nuclei were not isolated, not counted, and the DNA was not estimated per nucleus.

An explanation for increased DNA in the nuclei of the livers of rats fed nutritionally poor diets, as found in these experiments, is not clear. A suggestion is found, however, in the work of Leuchtenberger

(*12*), who found with Sarcoma 180 that in the early stages of pycnosis there was a considerable increase of DNA in the nuclei. It may be possible that under inanition liver nuclei enter a similar condition, and under continued stress many of the nuclei may pass through severe pycnosis and disappear, thus reducing the number of nuclei in the liver.

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A Suggested Simplification of Blood Volume Analysis Using the Dye T1824

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The analysis for blood volume as initially practiced (*1*) involved drawing a "blank" blood specimen, injection of the dye, and the withdrawal of four additional dyed blood specimens at noted time intervals. Subsequent workers (*2, 3*) established that satisfactory results were obtainable through the use of a "blank" blood, injection of the dye, and blood withdrawal to obtain a "dyed" specimen 10 min after injection of the dye.

The use of the plasma blank, although theoretically correct, is of questionable value in view of problems arising from differences in turbidity and/or degree of hemolysis (*1, 3–5*) between the blank and dyed specimen.

The observation (*3*) that the absorbency of the dye T1824 in plasma at 680 mμ is one fourth the absorbency at 620 mμ has suggested the following experiment designed to demonstrate that the blank specimen is not required.

Pooled plasma specimens were prepared to contain known amounts of the dye T1824, and the samples were blanked out against the same undyed plasma specimen, other undyed plasma specimens, and 0.9% saline containing 2 ml plasma/100 ml (Table 1). The optical density at 680 was subtracted from the optical density at 620, and the result multiplied by 4/3, yielding the corrected optical density that could be equated to μg dye/10 ml plasma:

$$OD = 4/3(OD_{620} - OD_{680}),$$

where OD is corrected optical density, and OD_{620} and OD_{680} refer to readings made at these respective wavelengths in mμ.

TABLE 1

RECOVERY OF THE DYE T1824 USING DIFFERENT BLANK PLASMAS OR SOLUTIONS

Sample No.	μg T1824 added to prepared sample	μg T1824 found			
		Undyed plasma blank	Plasma A blank	Plasma B blank	0.9% saline containing 2% plasma
1	18.8	18.9	18.9	21.3	20.6
2	31.3	32.4	31.5	31.8	31.2
3	37.5	38.7	38.2	38.2	37.0
4	50.0	50.5	51.8	50.5	49.8

It is advantageous to use a plasma blank, rather than a "water" blank, since readings can be made in the most accurate range of the spectrophotometer scale.

Using this technique, the procedure for determining blood volume is thus simplified to injecting the dye and obtaining a single blood specimen at a noted time interval.

Since in time of an emergency particularly, many of the patients whose blood volumes are required are receiving blood, plasma, or other fluids parenterally, the injection of the dye could be made through the tubing connecting the fluid reservoir with the vein. In this form the blood volume analysis is simplified to the point where the patient need be approached but one time to obtain a single "dyed" blood specimen, which can be used to establish the required hematocrit reading as well.

Interesting details concerning the variations in blood volume during parenteral therapy could be established by taking blood samples subsequent to the administration of the dye, using the correction factor established for excretion of dye (*3*).

$$OD(\text{zero time}) = OD_t(1 + .00187t),$$

where OD_t is optical density of the specimen, obtained as above, and t = time in minutes after the injection of the dye.

The blood volume is readily calculated from the optical density at zero time by familiar procedures.

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