5.2 leaves per plant whereas the control plants had 4.6 leaves per plant. The height of the plants from the ground level was measured. Table II gives the height of the tallest and shortest plants in each group, as well as the average height of all plants in each group in inches. The total weight of plant tissue above ground just as removed from the control flat was 60.9 g and from the treated flat 81.7 g. It is obvious from the foregoing results that the stimulatory effects of terramycin upon the germination and subsequent growth of sorrel plants caused a difference which was still apparent after 46 days.

A group of 49 sweet corn seeds was planted in each of 2 greenhouse flats: one control, one experimental. At the end of 4 weeks the plants were removed from the soil. Of the 49 seeds in the treated flat, 20 had germinated and formed a plant. Of those in the control flat, only 12 had germinated. The height of each plant from ground level was measured. Table II gives the height of the tallest and shortest plant in each group, as well as the average height of all plants in each group in inches. The total weight of plant tissue above ground just as removed from the control flat was 23 g and from the treated flat 45 g. The combined plant tissue from each group was dried at 105°C to a constant weight. The dry weight of all plants in the control group was 2.4 g and in the treated group it was 5.1 g.

Summary. Data from 3 types of experiments have shown a stimulation of plant growth by antibiotics: tissue culture, standard laboratory seed germination, and seed germination and subsequent growth in soil. The use of antibiotics in animal nutrition, particularly in the feeding of swine and poultry, is already prevalent. The preliminary experiments here described suggest the possibility that antibiotics might have a practical application in the stimulation of plant growth as well as in the control of plant diseases(7-9). The use of tissue culture technics should prove a useful tool in the study of the mechanism of stimulatory action by antibiotics.

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Intracellular Distribution of Estrogen Inactivating Mechanism in Rat Liver and Other Tissues.* (19711)

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The role of the liver in the inactivation of estrogens has been studied extensively for various species. A comprehensive review of the literature was presented by Paschkis and Rakoff(1). DeMeio and coworkers(2) and Coppedge *et al.*(3) demonstrated the importance of adding disphosphopyridine nucleotide (DNP) and nicotinamide when using male rat liver mince, as contrasted to liver slices, for the inactivation of alpha-estradiol. The object of the study here described was to

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localize the enzyme factors concerned with the inactivation of natural and synthetic estrogens through fractionation of rat liver homogenates by differential centrifugation.

Materials and methods. Female rats of the Holtzman-Rolfsmeyer strain, 50 days of age and weighing 130-140 g, were the source of tissues fractionated. Young adult female mice (Carworth Farm CF1 strain), weighing 17-22 g, were used for estrogen assay.

The rats were killed by cervical dislocation and a 10% homogenate of liver or other tissue prepared in 0.25 M sucrose using a cone-shaped glass homogenizer which was cooled in an ice bath. The homogenate was fractionated in the high speed attachment of an International refrigerated centrifuge at a temperature of 3-4°C essentially as described by Schneider(4) except that the nuclei in most cases were washed once only. The fractions obtained with this technic were nuclei, mitochondria, microsomes and supernatant.

Aqueous solutions of the hormones were prepared in phosphate-saline buffer according to Lehninger and Scott(5) except for the omission of glucose. The hormones tested were estradiol-17 β (International hormones), estrone (International hormones), and diethylstilbestrol (The Matheson Co.). The riboflavin-5′-phosphate (monosodium salt) was from Hoffmann-LaRoche, Inc.

The *in vitro* inactivation‡ of the hormones was carried out in 20 cc test tubes as follows: To each tube was added the hormone solution (pH 7.25) containing 3.75 μg of estradiol, 4.7 μg of estrone or 4 μg of diethylstilbestrol, and enough phosphate-saline buffer (pH 7.25) to give a final volume of 6 cc per tube after addition of nicotinamide, DPN and tissue. Just prior to incubation nicotinamide and DPN were introduced to give final concentrations of 0.05 M and 0.0005 M, respectively, and the homogenate or fraction(s) of liver or other tissues tested was added. The quantity of tissue used is expressed as milligrams-equivalent (mg.eq) which denotes the weight of original fresh tissue from which the fraction was obtained. The tubes were incubated for 2 hours at 38°C and then heated for 10 minutes on a boiling water bath to prevent further enzymatic activity.

The estrogenic activity was determined by the uterine weight method using mice ovariectomized 10 to 14 days prior to injection. Four mice were used per dose, each mouse receiving 0.1 cc of the incubation mixture subcutaneously twice daily for 3 days. The incubation mixture was injected without extraction or other treatment. The mice were autopsied 72 hours after the first injection, any fluid expressed from the uteri, and uterine and body weights recorded.

Each incubation mixture had a volume of 6 cc and the quantity injected per assay mouse was 0.6 cc. Thus each mouse received 1/10 of the incubation mixture including the residuum from 0.375 μg estradiol, 0.47 μg estrone or 0.4 μg diethylstilbestrol.

As standard procedure each experiment contained 2 controls. The first consisted of all the components except tissue and was, therefore, a measure of the sensitivity of the assay mice. The second control used each time contained 30 mg.eq. of liver homogenate per tube in addition to the components of the first control. The results obtained with this control measured the estrogen inactivating capacity of the liver of the particular rat whose tissues and tissue fractions were being tested.

In earlier experiments a comparison was made between the first control and one to which previously boiled homogenate was also added. With estradiol, the uterine weights obtained in both were quite comparable. With diethylstilbestrol, however, this is not the case as will be described later. Therefore, the non-tissue control was selected for these experiments.

The uterine weights of both controls varied somewhat from experiment to experiment. The results obtained are given both in absolute (uterine weight) and in relative terms (degree of inactivation).

Results and discussion. The results obtained with estradiol are summarized in Table I, with estrone in Table II and with diethyl-

‡ "Inactivation" as used in this paper refers to loss of biological activity of the hormone as measured by its effect on the uteri of ovariectomized mice.

TABLE I. Inactivation of Estradiol by Homogenates and Fractions of Rat Liver and Other Tissues.

Fraction*	Amt/mouse, mg.eq.†	No. exp.	Mean uterine wt, mg (range)	Relative degree of inactivation, % (range)
Estradiol alone	.375 μg	12	106 (86–133)	0
Hom	3	12	39 (25–57)	100
Hom	6	6	41 (33–51)	94 (78–113)
Hom	15	1	80	38
Boiled Hom	3	2	94 (91–96)	2 (-11–15)
N	6	1	102	9
N	12	2	78 (67–85)	12 (7–16)
Mt	12	2	88 (85–91)	25 (-3–61)
Mt	20	2	88 (83–94)	10 (6–15)
Mc	12	8	88 (65–114)	27 (-3–48)
S	8	2	74 (62–85)	18 (15–21)
S	12,14	2	86 (75–98)	40 (36–43)
Mt 6 and S	6	1	89	−8
Mt 12 " S	8	2	83 (63–103)	37 (31–43)
Mc 6 " S	6	5	52 (31–78)	93 (66–114)
Mc 12 " S	8	9	33 (17–55)	109 (93–122)
Mc 12 " RMP	4.5 μg	3	49 (36–56)	86 (81–91)
Mc 12 " RMP	9 μg	1	41	109
RMP	9 μg	2	74 (71–76)	12 (-5–28)
K–Hom	3	1	103	−25
K–Mc 12 and K–S	8	1	90	−4
K–Mc 12 " L–S	8	1	91	−5
L–Mc 12 " K–S	8	1	18	112
SG–Hom	3	1	81	23
SG–Mc 12 and SG–S	8	1	84	20
SG–Mc 12 " L–S	8	1	76	30
L–Mc 12 " SG–S	8	1	37	85

* Hom = homogenate; N = nuclei; Mt = mitochondria; Mc = microsomes; S = supernatant; RMP = riboflavin monophosphate; K = kidney; SG = salivary glands; L = liver.
† mg.eq. denotes the wt of original fresh tissue from which the fraction was obtained.

TABLE II. Inactivation of Estrone by Homogenates and Fractions of Rat Liver.

Fraction*	Amt/mouse, mg.eq.†	No. exp.	Mean uterine wt, mg (range)	Relative degree of inactivation, % (range)
Estrone alone	.47 μg	4	102 (74–112)	0
Hom	3	4	40 (22–50)	100
Hom	6	2	46 (39–52)	95 (93–96)
N	12	2	73 (71–75)	2 (-2–5)
Mt	20	2	102 (88–117)	13 (-13–39)
Mc	6	1	103	16
Mc	20	2	95 (89–101)	26 (18–34)
S	12,20	2	102 (101–102)	15 (13–17)
Mt 12 and S	12	2	96 (89–103)	24 (9–39)
Mc 6 " S	6	2	52 (42–61)	97 (81–113)
Mc 12 " S	12	3	37 (25–49)	108 (95–125)

* Hom = homogenate; N = nuclei; Mt = mitochondria; Mc = microsomes; S = supernatant.
† mg.eq. denotes the wt of original fresh tissue from which the fraction was obtained.

stilbestrol in Table III. All results are expressed in terms of hormone and tissue administered per mouse, but the amounts incubated *in vitro* in each case were 10 times this quantity, as indicated above.

The relative degree of inactivation was computed separately for each experiment. The mean uterine weight obtained with the incubation mixture containing estrogen but no tissue was designated as 0% inactivation. The mean uterine weight obtained on incubating the estrogen with 3 mg.eq. of liver homogenate was arbitrarily designated as 100% inactivation. All other uterine weights obtained in a

TABLE III. Inactivation of Diethylstilbestrol by Homogenates and Fractions of Rat Liver and Other Tissues.

Fraction*	Amt/mouse, mg.eq.†	No. exp.	Mean uterine wt, mg (range)	Relative degree of inactivation, % (range)
Dieth. alone	.4 μg	10	85 (63–114)	0
Hom	3	10	22 (17–28)	100
Hom	6	1	22	103
Hom	15	1	19	106
N	6	1	48	41
Mt	6	1	52	32
Mt	12	3	70 (62–90)	26 (13–39)
Mc	6	2	57 (52–62)	42 (32–52)
Mc	12	4	41 (27–68)	76 (51–93)
S	3	3	35 (32–38)	83 (77–88)
S	6	5	27 (24–34)	92 (67–99)
S	12	3	25 (20–30)	98 (93–106)
Mc 6 and S	6	4	25 (20–27)	100 (97–107)
Mc 12 " S	12	3	26 (24–28)	97 (95–100)
RMP	4.5 μg	2	21 (19–24)	104 (96–110)
RMP	9 μg	5	24 (20–28)	96 (86–107)
K–Hom	3	1	92	13
K–Mc	6	1	109	−8
K–S	6	1	104	−2
Boiled L–Hom	6	5	25 (19–39)	86 (62–98)
" L–N	6	1	33	69
" L–Mc	6	1	48	40
" L–S	6	2	74 (74–74)	−20 (−30–−9)

* Hom = homogenate; N = nuclei; Mt = mitochondria; Mc = microsomes; S = supernatant; RMP = riboflavin monophosphate; K = kidney; L = liver.

† mg.eq. denotes the wt of original fresh tissue from which the fraction was obtained.

given experiment were expressed in terms of the control figures for that experiment. Most of these weights ranged between the uterine weight representing 0 and that representing 100% inactivation. Values less than zero mean that the uteri showed greater stimulation than was produced with estrogen alone. Values above 100 indicate that the inactivation was greater than that obtained when the estrogen was incubated with 3 mg.eq. of homogenate.

The formula used for each experiment to calculate the degree of inactivation is $\frac{C_1—X}{C_1—C_2}$ where C_1 is the mean uterine weight obtained with hormone alone, C_2 the mean uterine weight with 3 mg.eq. of liver homogenate, and X the mean uterine weight of each of the other experimental groups tested against the above controls. One of the experiments included in Table I is listed below with its controls to illustrate the method used (the abbreviations are explained at the bottom of Table I):

Fraction	Amt/mouse, mg.eq.	Mean uterine wt, mg	Relative degree of inactivation, %
Estradiol alone (C_1)	.375 μg	89	0
Hom. (C_2)	3	28	100
Mc	12	81	13
Mt	12	91	−3
Mc 12 and S	8	23	108
Mt 12 and S	8	63	43
Mc 12 and RMP	4.5 μg	36	87

Maximum inactivation of estradiol and estrone by liver homogenate occurred when 3 mg.eq. was used. At this concentration of tissue some activity remained as indicated by a uterine weight of 40 mg compared with 24 mg for ovariectomized controls. Six mg.eq.

of homogenate caused slightly less inactivation and in one experiment with estradiol 15 mg.eq. of homogenate gave only 38% as much inactivation as 3 mg.eq. This is in agreement with the finding of Segaloff(6) that with increasing amounts of liver and a standard amount of estradiol increasing instead of decreasing amounts of recoverable estrogenic activity are obtained. This inverse relationship did not obtain with the liver fractions since values above 100% were obtained in some instances with 12 mg.eq. The inactivation of diethylstilbestrol showed no decrease whether 3, 6, or 15 mg.eq. of homogenate was used.

Three mg.eq. of liver homogenate reduced the biological activity of 0.375 μg of estradiol as shown by the reduction in uterine weights from 106 mg to 39 mg (Table I). None of the 4 liver fractions (nuclei, mitochondria, microsomes and supernatant) produced a comparable reduction in activity when used alone in concentrations up to 20 mg.eq. On recombining various fractions it was found that microsomes and supernatant gave inactivation equivalent to that produced by homogenate. With a combination of 6 mg.eq. of each, the inactivation of estradiol was 93% of that obtained with homogenate, and 12 mg.eq. of microsomes and 8 of supernatant gave 109% inactivation.

The factor in the microsome fraction was found to be heat-labile, that in the supernatant relatively heat-stable(7). This suggests that the enzyme(s) is associated with the microsomes and the cofactor(s) with the supernatant.

The supernatant can be replaced by riboflavin monophosphate (RMP). With 12 mg.eq. of microsomes, 4.5 μg of RMP caused 86% inactivation of estradiol and 9 μg of RMP 109% inactivation. This finding may be of interest in view of the deleterious effect of dietary deficiencies on the *in vivo* estrogen inactivating mechanism, reported variously as caused by vit. B deficiency(8,9) or inanition(10,11). Further work on this aspect is needed.

Rat kidney and salivary gland were fractionated and tested to determine whether the inactivating capacity was specific for liver fractions. Neither the homogenate nor the combined microsome-supernatant fractions of kidney or salivary gland inactivated estradiol significantly. When *liver supernatant* was added to the microsomes of either kidney or salivary gland, there was also no significant reduction in activity. However, the addition of kidney or salivary gland supernatant to *liver microsomes* gave results comparable to those obtained with the 2 liver fractions. This indicates that the estradiol-inactivating enzyme(s) found in the liver microsomes is absent in the comparable fraction of the other tissues tested. The necessary cofactor(s), however, seems to be more generally distributed since the supernatant of kidney or salivary gland could be substituted for liver supernatant. The properties of the supernatant are being investigated further at the present time.

Table II summarizes the results obtained when various liver fractions were incubated with estrone. As with estradiol none of the 4 fractions was as effective as homogenate in reducing the biological activity of estrone. When the microsomes and supernatant were combined, 97% inactivation occurred with 6 mg.eq. and 108% with 12 mg.eq.

In most of the experiments with estradiol and estrone the nuclear and microsome fractions were washed only once. In some of these the inactivation obtained was appreciable although still well below that produced by the combined microsome-supernatant fractions. A comparison was therefore made of the various fractions before and after one and two washings. In each case the washings removed most of the inactivating capacity and the washed microsomes were still as effective as the unwashed fraction when combined with the supernatant.

The ability of homogenate and various fractions to inactivate diethylstilbestrol is summarized in Table III. Three mg.eq. of liver homogenate completely inactivated 0.4 μg diethylstilbestrol. This quantity of homogenate was more effective with diethylstilbestrol than with the natural estrogens. A possible explanation is that the conversion products of estradiol and estrone retain some estrogenic activity following incubation with

liver(12), whereas those of diethylstilbestrol appear to be inactive(13).

Liver supernatant inactivated 83%, 92% and 98% of the diethylstilbestrol at concentrations of 3, 6 and 12 mg.eq., respectively. The addition of microsomes had almost no effect in contrast to the results obtained with the natural estrogens.

The value of 76% obtained with 12 mg.eq. of microsomes was possibly due in part to incomplete removal of supernatant from the submicroscopic particles inasmuch as this fraction had not been washed in these particular experiments. A non-enzymatic factor may also be involved and will be discussed later.

When kidney tissue was incubated with diethylstilbestrol, none of the fractions tested inactivated the hormone. Thus in the inactivation of diethylstilbestrol the supernatant fraction is the only one which is necessary and apparently must be from the liver. However, with estradiol the microsomes must be from the liver but the supernatant can be from other tissues. Other differences between the inactivating mechanisms for these 2 estrogens are being studied further, including requirement for DPN, heat lability, etc.

Four and one-half or 9 μg of RMP inactivated diethylstilbestrol in the absence of any tissue. Thus RMP is able to replace liver supernatant in inactivating estradiol and diethylstilbestrol, in the latter case acting alone (a non-enzymatic reaction), in the former in conjunction with microsomes.

Incubation of *boiled* liver homogenate with diethylstilbestrol resulted in the loss of 86% of the estrogenic activity, which indicates that a non-enzymatic mechanism in the liver can also inactivate diethylstilbestrol. Boiled nuclei and boiled microsomes destroyed 69% and 40% respectively of the activity. It is quite possible, therefore, that the relatively high values reported in Table III for the unboiled nuclear and microsome fractions are due mainly to a non-enzymatic mechanism. The inactivation of diethylstilbestrol by supernatant appears to be enzymatic, however, since this fraction after being boiled completely loses its effectiveness.

Summary. Rat liver homogenate was separated into nuclei, mitochondria, microsomes and supernatant fractions by differential centrifugation. The homogenate, the fractions and combinations of fractions were studied for their ability to decrease the activity of estradiol, estrone or diethylstilbestrol when incubated at 38°C for 2 hours in the presence of DPN and nicotinamide. The uteri of ovariectomized mice were used to determine the amount of biological activity remaining.

No single fraction decreased the activity of estradiol markedly. The microsomes and supernatant, when recombined, were comparable to homogenate in inactivating this hormone.

Though the homogenate of kidney or salivary glands was inert in this respect, the supernatant of either tissue when combined with liver microsomes effectively inactivated estradiol. The supernatant could also be replaced by riboflavin monophosphate (RMP). The microsomes of kidney or of salivary glands when used with liver supernatant failed to inactivate estradiol. The inactivation of estrone occurred with the same liver fractions necessary for estradiol: microsomes and supernatant. No appreciable inactivation occurred with any single fraction. For the *in vitro* inactivation of diethylstilbestrol, liver supernatant alone was almost as effective as homogenate. Kidney supernatant was ineffective. RMP alone, in the absence of any tissue, destroyed the estrogenic activity of diethylstilbestrol. A non-enzymatic inactivation of this hormone by liver tissue was also indicated.

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Effect of Cystine and Methionine on Healing of Experimental Wounds.* (19712)

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In previous reports(1,2), the more rapid rate of healing of experimental wounds in animals fed a high protein diet as compared to those on a lower protein diet was shown to be due to the greater intake and retention of protein sulfur. It was also shown that methionine could serve as the source of protein sulfur. This explained earlier work which had indicated that methionine increased the rate of healing(3-5). Other essential amino acids (6,7) were found to have no effect on the *healing index,* a numerical measure of a function which is proportional to the rate of healing.

Methionine may have 2 possible roles, involving its sulfur atom, which might affect the *healing index.* First, it might be required directly in the healing processes for such reactions as protein synthesis. Secondly, methionine might serve as a precursor for some other sulfur-containing compound required during healing. Although these alternatives need not necessarily be mutually exclusive, it is not unreasonable to presume that one function will be more important than the other.

In the present paper, it is shown that the *healing index* can be affected by cystine to the same extent as by an equivalent amount of methionine, on the basis of sulfur.

Experimental. The experiments to be described were carried out in a similar manner to those previously reported(1,2). In each experiment, 3 groups of 24 female albino rats (200 ± 20 g) were maintained on a basal diet for 5 days prior to wounding. The basal diet consisted of 6 g casein, 10 g lard, 2 g corn oil, 5 g salt mixture(8), 77 g sucrose, 1500 I.U. vit. A,† 210 I.U. vit. D,† 1 mg thiamine HCl, 1 mg riboflavin, 1 mg pyridoxine HCl, 15 mg nicotinic acid amide, 4 mg calcium pantothenate, 0.5 mg 2-methyl naphthoquinone, 5 mg inositol, and 25 mg choline chloride. This diet would permit only a small amount of protein accretion in normal unwounded animals (as measured by nitrogen excretion and increase in body weight). On the day of wounding, the animals were transferred to the experimental diets. All the animals were given the same weighed amount of diet daily, in quantities which would be completely consumed. Distilled water was permitted *ad libitum.*

Standard experimental wounds were made on the back of the neck of the rats as previously described(1). At approximately weekly intervals, 1/3 of the animals in each group were sacrificed and the tensile strength of a number of 0.5 cm sections of the healing wound were measured(1,9). The relationship of tensile strength to time results in a curve which may be considered to be essentially a straight line. This line can be represented by the equation $T = kt + C$, where T is the tensile strength in grams, and t, the time in days. C is a constant. The slope of this line (K) is the *healing index,* and may be computed from the equation:

$$K = \frac{T_2 - T_1}{t_2 - t_1}$$

where T_1 is the tensile strength at time t_1, and T_2 is the tensile strength at time t_2. K

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† From oleum percomorphum.