given these classical titles, as the writers wish to show how each subject is of importance to mankind. Thus, the part on chemistry is called "Matter as Organized Energy Possesses Properties Which Are Indispensable to Man," and that on astronomy "Man's Increased Knowledge of the Cosmos Has Modified His Thinking." An integrated thread of continuity is in this manner woven through somewhat diverse material, and the bearing of all the sciences on man's thought and activities is developed.

In the first section the errors of ancient concepts are described and the scientific method explained. Some of the more recent findings of astronomy are set forth. From this, the authors proceed to demonstrate how superstitions have been demolished by knowledge, and how practical use is made of astronomy in determinations of time and geographical location. The second section explains how the distinctions between matter and energy are broken down, and the third describes the many possible chemical rearrangements of matter, including a chapter on organic chemistry and one on the way chemical products add to the conveniences and pleasures of living.

The fourth unit discusses the physics of the various forms of energy and shows how all may be applied. The fifth reviews the effects of meteorology on life. The final part of the book is devoted to a discussion of geology, starting with a review of the structure of the earth and showing how geological changes are continually in progress. The evolution of the present surface conditions of the earth is traced and finally the authors treat the practical importance of geological forces which make many mineral products available to mankind.

As is inevitable in a book jointly written by several authors, there are differences in quality between the several sections. Thus, for example, in the chapters on geology the illustrations are referred to in the text and serve to illuminate the subject-matter there discussed, while in those on astronomy and physics the photographs and diagrams are too often left for the instructor to explain. Nor is the choice of illustrations in these sections as good. In some cases, as on page 83, they are more complex than necessary and contain material requiring explanations not germane to the matter under discussion. Further explanations by the instructor will be necessary on page 272, where it is stated that energy is kinetic or potential, and the student is left to wonder just how heat energy and subatomic energy are to be fitted into these two categories. On the whole, this reviewer was left with the distinct impression that the authors of the parts on geology and chemistry were better able to expound their subjects in a clear and interesting manner which covered the essential points than were those on physics and astronomy. On the other hand, the sections on physics and astronomy do contain many simple and instructive treatments such as that on latent heat. The scientific method is both praised and explained, but the student is given little instruction in its use and few opportunities to practice it himself. It would seem proper to add some problems for quantitative computation to the admirable ones calling for general discussion.

This text should appeal to the average student who did not expect to study science beyond a survey course. The writers of this book have undoubtedly succeeded in presenting the results of recent investigations to non-scientists in an interesting manner, while, at the same time, explaining how these results are of importance to mankind.

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SPECIAL ARTICLES

PERFUSION OF RAT LIVERS WITH ESTROGEN IN VITRO[1]

THE role of the liver in metabolism of steroid hormones has been a matter of interest for some time. Since the experiments of Silberstein *et al.*[2] and of Zondek[3] on the incubation of natural estrogens with liver brei the original finding that the free hormone is rapidly inactivated has been generally substantiated. Confirmation has been attained by a variety of direct and indirect approaches, including: (a) damage of the liver of rats by administration of CCl_4 and the observation of increased end-effects of both endogenous[4] and exogenous estrogen;[5] (b) demonstration of the ineffectiveness of estrogen crystals or pellets placed in sites drained by the hepatic portal systems[6,7] with similar inactivation on direct injection into the spleen;[8]

[1] Aided by grants from G. D. Searle and Company and the National Research Council Committee for Problems of Sex.

[2] F. Silberstein, P. Engel and K. Molnar, *Klin. Wochsch.*, 12: 1693, 1933.

[3] B. Zondek, *Skand. Arch. Physiol.*, 70: 133. 1934.

[4] N. B. Talbot, *Endocrinol.*, 25: 60, 1939.

[5] G. Pincus and D. W. Martin, *Endocrinol.*, 27: 838, 1940.

[6] G. R. Biskind and J. Mark, *Bull. Johns Hopkins Hosp.*, 65: 212, 1939.

[7] G. R. Biskind, *Endocrinol*, 28: 894, 1941; *Proc. Soc. Exp. Biol. Med.*, 47: 766, 1941.

[8] A. Segaloff and W. O. Nelson, *Proc. Soc. Exp. Biol. Med.*, 48: 33, 1941.

(c) perfusion of a heart-lung-liver preparation (dog) with estrone and the observation of inactivation in a few minutes with little or no inactivation by a control heart-lung preparation;[9] (d) incubation of various estrogens with liver slices.[10,11,12] Experiments involving the administration of estrogen and the isolation of excretion products from the urine have demonstrated that *in vivo* estradiol and estrone are intraconvertible in test animals and men, and that estrone is converted in large part to estriol by human male subjects (for a recent review see Doisy *et al.*,[13] Pincus and Pearlman,[14] also Schiller and Pincus).[15] The probabilities of such conversion by the liver have been investigated only by the experiments of Heller,[10,11] who obtained evidence for an apparent irreversible destruction of α-estradiol by rat liver slices and a probable conversion of estrone to α-estradiol which is rapidly destroyed.

We have undertaken the perfusion of intact rat livers with α-estradiol dissolved in a perfusion medium usually consisting of a mixture of Ringer-Locke solution and defribinated rat blood (see Table 1). Under aseptic conditions female adult rats in proestrus or estrus were rapidly bled, the liver *in situ* was then washed through with Ringer-Locke solution, dissected and cannulated through the portal vein, care being taken that the cannula contained Ringer-Locke solution to prevent air-embolism formation. The cannulated specimen was mounted in a perfusion apparatus having a pulsating flow of 70 beats per minute with a pressure at the portal vein sufficient to ensure a flow through the organ of 3 cc of aerated medium per minute (approximately 120 mm Hg). The entire apparatus was incubated at 37° C.

In order to recover the estrogens from the perfusing medium the following extraction was employed: (a) addition of an alcohol-ether mixture (3 parts 95 per cent. ethyl alcohol to one part ethyl ether) using 18 cc for each cc of blood or serum in the medium; (b) washing the precipitate formed with 95 per cent. ethyl alcohol using one-fourth to one-half the volume of the original alcohol-ether mixture; (c) the clear filtrates of (a) and (b) are combined, the ether and alcohol evaporated off and the watery residue extracted thoroughly with ethyl ether; (d) the ether extract is washed with 1 N NaOH to remove the estrogens, which are concentrated by acidifying the NaOH, extracting again with ether and evaporating the water-washed ether extract to dryness. An aliquot of this phenolic fraction is assayed with spayed rats by a modified Allen-Doisy method.[16] Separation of the phenolic extract into three fractions segregating principally estradiol, estrone and estriol respectively, is accomplished by methods previously described.[17,18] Hydrolysis of the perfusate when performed was accomplished by autoclaving at 14 pounds pressure for twenty minutes at pH 1–2.

Since it has been adequately demonstrated by Zondek[19] that rat blood exerts no effect on added estrogen, we have contented ourselves with a single one-hour perfusion through the apparatus of 3,200 r.u. of α-estradiol with perfusing medium alone (20 cc defibrinated blood plus 100 cc Ringer-Locke solution). We recovered 3,000 r.u., the 200 r.u. lost probably representing losses in manipulation although it is within the limits of error of our assay method (± 15 per cent.). Similarly 300 r.u. of α-estradiol was recovered completely in two incubations of 3 and 17 hours, respectively, with rabbit serum. Rabbit serum itself extracted by our methods contains no detectable estrogenic activity (less than 1 r.u. in 18 cc).

In Table 1 we present data from typical perfusion experiments. They demonstrate: (a) that with the larger amounts of α-estradiol the total activity of the perfusing fluid disappears rapidly, being reduced to less than one third in three hours (experiments 1, 2, 3, 4) and to approximately one sixth in six hous (experiments 2 and 4); (b) that this loss of activity is principally due to the formation of the less potent estrogens estrone and estriol (experiments 1 and 2); (c) that with smaller amounts of perfusing α-estradiol the loss of activity is more rapid (experiment 5) being practically complete on perfusion with 208 r.u. for 5 hours (experiment 6) since the liver perfused with medium alone yields a similar small unitage (experiment 7); (d) that acid hydrolysis of the three-hour (experiment 3) or six-hour (experiment 4) perfusate fails to increase the total activity, nor does the hydrolysate obtained enhance or diminish the activity of added α-estradiol (experiment 3); (e) that perfusion of the rat heart for three hours results in no conversion of perfusing α-estradiol, since the recovery observed in experiment 8 is practically all in the

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[13] E. A. Doisy, S. A. Thayer and J. T. Van Bruggen, *Federation Proc.*, 1: 202, 1942.

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[17] G. Pincus and W. H. Pearlman, *Endocrinol.*, 31: 507, 1942.

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TABLE 1

THE RECOVERY OF ESTROGENIC ACTIVITY FROM THE PERFUSING MEDIUM IN VARIOUS PERFUSIONS OF RAT ORGANS *in vitro* WITH α-ESTRADIOL

Experiment No.	Organ perfused	Perfusion medium	Amount of α-estradiol in perfusate (r.u.)	Perfusion time (hours)	Activity recovered from perfusate (r.u.)* Total	Weak phenolic non-ketonic (estradiol)	Weak phenolic ketonic (estrone)	Strong phenolic (estriol)	Remarks
1	Liver	25 cc defibrinated blood, plus 100 cc Ringer-L.	3200	3	1000	400	400	200	
2	"	20 cc defibrinated blood, plus 100 cc Ringer-Locke	3200	6	570	220	220	132	An aliquot taken at 3 hrs. assayed 1000 r.u.
3	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	4080	3	1200† 1000‡	...	...	...	¼ of total plus 416 r.u. of α-estradiol assayed 700 r.u. ¼ of total acid hydrolyzed plus 416 r.u. α-estradiol assayed 730 r.u.
4	"	80 cc rabbit serum plus 15 cc Ringer-Locke	1664	6	134† 128‡	...	...	...	
5	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	300	3	43	...	...	...	
6	"	20 cc defibrinated blood, plus 100 cc Ringer-Locke	208	5	15	...	...	...	
7	"	25 cc defibrinated blood, plus 100 cc Ringer-Locke	0	3	14	...	...	...	
8	Heart	50 cc rabbit serum plus 75 cc Ringer-Locke	300	3	270	270	4	0	

* By our assay method 1 r.u. = 0.125 microgram α-estradiol, 1.0 microgram estrone, 1.0 microgram estriol.
† By routine extraction (see text).
‡ After acid hydrolysis (see text).

estradiol fraction, the small amount (4 r.u.) in the estrone fraction being accountable to fractionation error.

These data controvert the findings of Heller[10] and Zondek[19] that α-estradiol is not converted to other estrogens by liver *in vitro*. It is possible that autolyzed tissue, as represented by liver slices or brei, may release substances (enzymes or oxidants) that are destructive, whereas the intact organ exerts no such effect. Macroscopic examination of the livers of these experiments gave no indication of retrogression or autolysis. Our data do indicate that the liver may normally be concerned in the conversion of α-estradiol to estrone and estriol observed in injection and urinary recovery experiments.[15, 20, 21]

A fractionation of the urine of male rats receiving estrone by injection indicates a similar conversion by the intact animal; furthermore, partially hepatectomized animals show less destruction of the exogenous estrogen and also less conversion than normal animals (Schiller, unpublished data).

It is notable that these data give no indication of estrogen detoxification by the perfused rat liver. Either conjugation occurs at a much slower rate than conversion or the participation of another organ is requisite. Alternatively, the conditions of our experiments result in a breakdown of a detoxifying mechanism in the liver.

Details of these and related experiments will be published elsewhere.

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INABILITY TO PASS PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS[1]

THE chief obstacle facing investigation of the etiology of primary atypical pneumonia (pneumonitis) has been the lack of suitable laboratory animals which regularly exhibit pneumonia after intranasal inoculation with throat washings, sputum or lung specimens from sick patients.

Stokes *et al.*[2] and Reimann[3, 4] among others have reported indifferent results after previously obtaining a passage virus. Recently Dingle *et al.*[5] have reported

[1] Published with the approval of the chief of the Bureau of Medicine and Surgery, United States Navy.
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[4] H. Reimann and W. Haven, *Arch. Int. Med.*, 65: 138, 1940.
[5] J. Dingle *et al.*, *War Med.*, 3: 223, 1943.