The Effect of 7-Ketocholesterol on the Rabbit

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Wintersteiner and Bergstrom found that a stream of air passed through a colloidal solution of cholesterol resulted in the production of principally 7-ketocholesterol and 7-hydroxycholesterol (*10*). Kendall, Meyer, and Bevans (*6*) reported that single intravenous injections into rabbits of such oxidized solutions produced lipid droplets within the cells of the intima in 24 hr. Multiple intravenous injections led to an immediate increase of sudanophilic material in the intima, which roughly paralleled the amount of oxidized material injected. In control experiments of these workers, early changes did not result from the administration of unoxidized cholesterol. However, if high plasma cholesterol levels were maintained for several weeks by repeated injections of either unoxidized or oxidized material, there was little difference in the extent or character of the lesions produced.

7-Ketocholesterol was prepared by us according to the method of Windaus, Lettre, and Schenk (*9*). Altschul (*1*) found that when this compound was given to rabbits either orally (in capsules of 0.3 g daily) or percutaneously, as a solution in benzene and vegetable oil (approximately 0.1 g daily), it produced none of the cellular reactions characteristic of the administration of pure cholesterol. However, 7-ketocholesterol administered orally or percutaneously had a definite effect on the liver. Examination showed atrophy of the liver cords and the presence of necrotic areas, numerous giant cells, and an overgrowth of connective tissue and biliary epithelium. In two instances, this organ showed a characteristic "hobnail" surface.

Thus the pathological changes brought about with 7-ketocholesterol did not parallel those found by Kendall *et al.*, using colloidal solutions of cholesterol oxidized according to Wintersteiner and Bergstrom, and therefore presumed to contain chiefly 7-ketocholesterol and 7-hydroxycholesterol. This difference in pathological findings suggested that the effect of administering a mixture of α and β epimers of 7-hydroxycholesterol should be studied, and this is at present under investigation. Also of possible significance is the observation that, under optimum conditions for oxidation, these colloidal solutions contained about 20% unchanged cholesterol (*10*).

Collier and Cox have found that percutaneous administration of approximately 0.1 g of 7-ketocholesterol daily for 64 days resulted in a greatly increased sterol concentration (calculated as cholesterol) in the plasma, as detected by a modification of the Tshugaev reaction (*8*). However, examination of plasma from an animal which received orally 0.3 g of 7-ketocholesterol daily for 40 days showed a comparatively normal sterol level. The plasma of both animals contained only a trace of 7-ketocholesterol, when determined by the micromethod of these workers (*2*). The possibility that liver selectively reduces 7-ketocholesterol to cholesterol, or to a sterol giving a color reaction similar to that of cholesterol when determined by the zinc chloride-acetyl chloride reagent, is now being examined. Of interest in this investigation is the observation that the epimeric 7-hydroxycholesterols, which can be prepared by the selective reduction of the keto group in 7-ketocholesterol (*9*), have been isolated from ox liver in recent years (*3*, *4*, *7*). However, it is not known whether these hydroxycholesterols are true intermediates in sterol metabolism, or only autoxidation products of cholesterol, perhaps formed during the process of extraction (*5*, *11*).

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References

1. Altschul, R. In press (Charles C. Thomas, publisher).
2. Collier, H. B. and Cox, R. H. *Fed. Proc.*, in press.
3. Haslewood, G. A. D. *Biochem. J.*, 1939, **33**, 709.
4. *Ibid.*, 1941, **35**, 708.
5. *Ibid.*, 1942, **36**, 389.
6. Kendall, F. E., Meyer, W., and Bevans, M. *Fed. Proc.*, 1948, **7**, 1, 273.
7. MacPhillamy, H. B. *J. Amer. chem. Soc.*, 1940, **62**, 3518.
8. Tshugaev, L. and Gastev, A. *Ber. deut. chem. gesellsch.* 1910, **42**, 4631.
9. Windaus, A., Lettre, H., and Schenck, F. *Ann. chem. Liebrig.*, 1935, **520**, 98.
10. Wintersteiner, O., and Bergstrom, S. *J. biol. Chem.*, 1941, **141**, 597.
11. Wintersteiner, O., and Ritzmann, J. R. *J. biol. Chem.*, 1940, **136**, 697.

Survey of Chinese Drugs for Presence of Antibacterial Substances[1]

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In view of recent studies on the production of antibiotic substances by higher plants (*1–6*), it seems interesting to make a survey of drugs commonly used in the practice of Chinese medicine for the presence of antibacterial substances. The so-called Chinese drugs are actually roots, stems, seeds, leaves, or flowers of various higher plants in a very dehydrated state (prepared by special methods). This paper reports the results of such a survey.

To 10–20 g of a drug cut in small pieces, 150 ml of distilled water was added, and the mixture was then boiled slowly for 2–3 hr or longer to a final volume of about 25 ml. (This is the customary way of preparing Chinese medicine, except that ordinary tap water is used instead of distilled water.) After preliminary filtering, the filtrate, which is really a concentrated water extract

[1] This work was carried out in 1945 before departure of the authors for the United States. Since the return of the senior author to China in 1947, some of the experiments were repeated and extended.

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TABLE 1

ANTIBACTERIAL ACTION OF CHINESE DRUGS*

Name of drug	Identified scientific name	*Staph. aureus*	*E. coli*
Ma Huang	*Ephedra sinica* Stapf. (stem)	0	0
Ma Tou Ling	*Aristolochia debilis* S. et Z. (fruit)	0	0
Si Sin	*Asarum sieboldii* Miq. (root)	0	0
Ta Huang	*Rheum palmatum* L. (or *R. officinale* Baillon) (root)	28–35	+
Peh Tou Ung	*Anemone chinensis* Bunge (root)	0	0
Mao Ken	*Ranunculus pensylvanicus* L. (leaf and fruit)	0	8–10
Huang Lien	*Coptis chinensis* Franch (stem and root)	17–20	0
Lien Chiao	*Forsythia suspensa* Vahl. (fruit)	0	0
Kou Teng	*Strychnos panicula* Champ. (stem)	0	0
Chang San	*Dichroa febrifuga* Lour. (root and leaf)	0	+
Hai Tung Pi	*Erythrina* sp. (bark)	16–24	0
Tu Chung	*Eucommis* sp. *ulmoides* Oliv. (bark)	0	0
Chai Hu	*Bupleurum falcatum* L. (root, stem, and leaf)	+	0
Ku Tsan	*Sophora flavescens* Ait. (var. Galegoides Hemsl.) (root)	0	0
Keh Ken	*Pueraria Thunbergiana* Benth. (root)	0	0
Tsao Chieh Chieh	*Gleditschia sinensis* Lam. (stem)	0	+
Huang Peh	*Phellodendron chinense* Schneid. (bark)	0	+
Ku Lien Tzu	*Melia Azedarach* L. (fruit)	+	0
Yuan Chih	*Polygala sibirica* L. (root and leaf)	0	+
Ta Chih	*Euphorbia pekinensis* Rupr. (root)	0	0
Pa Tou	*Croton Tiglium* L. (seed)	0	0
Ta Fung Tzu	?	0	0
Shih Chun Tzu	*Quisqualis indica* L. (fruit)	0	0
Peh Chih	*Angelica sp.* (root)	0	0
Fang Fung	*Siler divaricatum* B. et H. (root)	0	0
She Chuang Tzu	*Selinum Monnieri* L. (seed)	0	0
Shan Shu Yu	*Cornus officinalis* S. et Z. (fruit)	18–24	0
Shih Nan	*Rhododendron indicum* Sweet (leaf)	+	0
Chai Tsao	*Lithospermum officinale* L. (root)	0	0
Poh Ho	*Mentha arvensis* L. (stem and leaf)	0	0
Ti Kuh Pi	*Lycium chinense* Mill. (bark and root)	0	0
Ti Huang	*Rehmannia glutinosa* Libosch (root)	0	0
Chu Tsien	*Plantago major* L. (stem and leaf)	0	0
Tsien Tsao	*Rubia cordifolia* L. (root)	10–14	0
Jen Tung Teng	*Lonicera japonica* Thunb. (vine)	0	0
Chieh Keng	*Platycodon grandiflorum* DC. (root)	0	0
Tsang Muh	*Atractylis ovata* Thunb. (root)	+	+
Kuan Tung	*Tussilago Farfara* L. (flower)	0	0
Pu Kung Ing	*Taraxacum mongolicum* Hand.-Mazz (leaf, stem, and root)	0	0
Niu Pang	*Arctium Lappa* L. (seed)	0	0
Chi Hsueh Teng	*Tripterygium Wilfordii* Hook. f. (vine)	12–16	0
Cheh Sish	*Alisma plantago* L. (root)	0	0
Pan Hsia	*Pinellia ternata* Breit. (root)	0	0
Peh Pu	*Stemona sessilifolia* Miq. (or *S. japonica* Miq.) (root)	0	10–12
Chih Mou	*Anemarrhena asphodeloides* Bunge (root)	+	0

* 0 = no activity + = very slight inhibition
Numerals indicate size of inhibition zone in mm

of the drug, was made bacteria-free by passing through a Mandler diatomaceous filter candle at 8-lb pressure, and then was tested against *Staphylococcus aureus* and *Escherichia coli.* (Both cultures were supplied by the Department of Bacteriology, School of Medicine, National Central University.) No attempt was made to make any of the assays quantitative. The test plates were prepared as follows: bacterial cultures were grown for 24 hr in nutrient broth and 1-ml portions of these suspensions were plated in 1.5% nutrient agar. Porcelain Peni-cylinders were affixed to the surface of these plates immediately after hardening of the agar and filled with the drug extract to be tested. The plates were incubated at 37° C for 18–24 hr. The diameters of the inhibition zones were measured and recorded in mm. Results of the test are given in the table.

From Table 1 it can be seen that six of the drugs, namely: Ta Huang (*Rheum palmatum* L. or *R. officinale* Baillon), Huang Lien (*Coptis chinensis* Franch), Hai Tung Pi (*Erythrina* sp.), Shan Shu Yu (*Cornus officinalis* S. et Z.), Tsien Tsao (*Rubia cordifolia* L. and Chi Hsueh Teng (*Tripterygium Wilfordii* Hook. f.) showed various degrees of antibacterial activity for *Staphylococcus aureus,* while two others; Mao Ken (*Ranunculus pensylvanicus* L.) and Peh Pu (*Stemona scssilifolia* Miq. or *S. japonica* Miq.) showed antibacterial activity (of relatively low potency) for *Escherichia coli.*

The eight drugs that showed bacterial inhibition were tested for their effect on the respiration of the respective bacteria and their toxicity to animal tissues. The effect on respiration was measured by using the Warburg type of microrespirometer and it was found that none of the drugs has any effect on bacterial respirations although bacterial multiplication was checked, indicating that the drugs are bacteriostatic in action. The toxicity was tested on living human leucocytes, following the general procedures used by the Oxford workers (*6*). The leucocytes continued to move for about 2–4 hr after the drug extracts were added. They are, therefore, nontoxic, which is to be expected, as prepared medicine is always administered orally in the practice of Chinese medicine.

References

1. CHEN, S. L., *et al.* *Nature,* Lond., 1945, **156**, 234.
2. LITTLE, J. E. and GRUBAUGH, K. L. *J. Bact.,* 1946, **52**, 587.
3. LUCAS, E. H. and LEWIS, R. W. *Science,* 1944, **100**, 597.
4. OSBORN, E. M. *Brit. J. exp. Path.,* 1943, **24**, 227.
5. SANDERS, A. G. Private communication, 1945.
6. SANDERS, D. W., WEATHERWAX, P., and MCCLUNG, L. S. *J. Bact.,* 1945, **49**, 611.