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REPORTS

Activity of Testosterone 5α-Reductase in Various Tissues of Human Skin

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In order to know the distribution of testosterone 5α-reductase activity in human skin, we developed a micromethod, in which we used 20–50 μg of various tissues microdissected from freeze-dried sections. The characteristics of this enzyme in the sebaceous gland are briefly described, as follows: the identified 5α-reduced metabolites are 5α-dihydrotestosterone, 5α-androstane-3β, 17β-diol and 5α-androstanedione; the optimal pH is about 7.5; and the apparent K_m is approximately 2.4×10^{-5} M.

The measurement of 5α-reductase activity of various components of the skin obtained from 7 men and 5 women revealed that the sweat gland (probably apocrine) in the axillary skin possessed the highest activity of 5α-reductase: the value was nearly 400 pmoles/mg dry weight/hr in the standardized condition. The sebaceous gland also showed a high activity of 85–261 pmoles/mg/hr. The hair follicles exhibited a significantly lower activity than the sebaceous gland. The enzyme activity was negligible in the epidermis, while it was detected in the dermis though the values determined were variable probably because of contamination with other components such as sweat glands and hair follicles.

Thus, the present study demonstrates that the 5α-reductase activity is mainly located in the apocrine sweat gland and sebaceous gland. This suggests that 5α-reduction of testosterone is an important step in mediating the action of androgens in these tissues.

Human skin has been shown to convert testosterone to 5α-dihydrotestosterone (DHT) and other 5α-reduced steroids both *in vivo* and *in vitro* [1–4]. Besides, in the androgen-dependent conditions like acne vulgaris, male pattern baldness and idiopathic hirsutism, an increased activity of 5α-reductase is observed [5–7]. These results are interesting in relation to the role of DHT in mediating the action of androgens in the target tissues such as the prostate [8,9]. However, from these studies with whole skin, it is not possible to know which components of the skin are responsible for the reaction, since human skin is composed of various tissues with different sensitivity to androgens such as epidermis, hair follicles, sebaceous glands, sweat glands and dermis, and the proportion of these constituents differs by regions and diseased conditions.

In an effort to overcome the problems outlined above, we devised a micromethod for determining 5α-reductase activity by use of 20–50 μg of tissues microdissected from freeze-dried skin. By this method, we quantitatively evaluated the 5α-reductase activity of various components of human skin, and confirmed the observation of Hay and Hodgins [10] that the enzyme activity was mainly located in apocrine glands and sebaceous glands.

MATERIALS AND METHODS

Preparation of Freeze-dried Tissues

Skin specimens were obtained at surgery from 7 men and 5 women (13–53 yr of age). A 5-mm punch biopsy was needed to obtain each specific tissue in the weight range of 20–50 μg. Specimens were immediately frozen in Dry Ice, cut into sections of 30 μ in thickness at −30°C in a cryostat and were vacuum-dried overnight. The sections were dissected into separate components under stereomicroscopy, magnification × 40, at room temperature, since the enzyme activity in the tissue sections did not differ whether they were handled in a cold room or at room temperature. For hair follicles, entire follicles except for medulla and cortex were dissected. The dissected tissues were weighed on a microelectric balance.

The possible effect of freeze-drying on the 5α-reductase was studied with a specimen of nevus sebaceus from subject D. When homogenate of fresh whole skin was used instead of freeze-dried, microdissected tissues, the rate of 5α-reduction was 6.76 pmoles/mg wet tissue/hr. Since the dry weight of tissues is about 30% of the wet weight and sebaceous glands weighed approximately 15% of this freeze-dried tissue (this specimen did not contain an appreciable amount of apocrine sweat glands), the activity is considered equivalent to 150 pmoles/mg dry tissue/hr, which does not differ significantly from the value of the freeze-dried tissue (Table II). The identified 5α-reduced metabolites formed by the fresh homogenate were same as those by the freeze-dried tissue.

Incubation

^{3}H-1,2-Testosterone (SA 42 Ci/mM, New England Nuclear Corp.) was purified by thin-layer chromatography before use with a suitable amount of unlabeled testosterone. The standard incubation mixture consisted of 2 μM (4×10^5 dpm) ^{3}H-1,2-testosterone, 5 mM glucose-6-phosphate, 1 mM NADP, 2 units/ml glucose-6-phosphate dehydrogenase, 100 mM Tris-HCl buffer, pH 7.5, and 10% propylene glycol, in a total volume of 50 μl. Each tube contained 20–50 μg of dissected tissues. Incubation was carried out at 37°C for 1 hr.

Extraction and Isolation of Steroid Metabolites

After incubation a 4-fold volume of chloroform-methanol (2/1:V/V) [11] containing 10 μg each of carrier steroids was added to the incubation mixture. The extracted steroids were spotted on precoated thin-layer chromatograph plates (Kieselgel 60 F_{254}, Merck). The plates were developed in a chloroform-methanol (99/1:V/V) solution as described by Gomez and Hsia [3]. Each of the bands representing the individual portions of the reference steroids was eluted with methanol. Aliquots of each eluate were transferred to counting vials containing 5 ml of 0.5% diphenyloxazole and 0.015% 5-phenyloxazole and assayed for radioactivity in a liquid scintillation spectrometer. The eluted steroids corresponding to DHT or androsterone and 5α-androstane-3α,17β-diol or 5α-androstane-3β,17β-diol were acetylated and analyzed by thin-layer chromatography with benzene-ethyl ether (8/2:v/v), and their purity was checked by recrystallization to a constant specific activity. The purity of the spot coinciding with 5α-androstanedione was also checked by recrystallization. The 5α-reductase activity was expressed by the sum of DHT, 5α-androstane-3β,17β-diol and 5α-androstanedione

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Abbreviations:
DHT: 5α-dihydrotestosterone

formed, since the radioactivity corresponding to other 5α-reduced metabolites such as 5α-androstane-3α,17β-diol and androsterone decreased to a negligible level after recrystallization in all of the tissue components (sebaceous glands, apocrine sweat glands, hair follicles, etc.). Blanks containing only the incubation mixture were run in each experiment.

DNA Determination

In some materials, DNA was determined by the method of Kissane and Robins [12] as modified by Santoianni and Ayala [13]. Fluorescence was assayed with a spectrophotometer (model 204, Hitachi).

RESULTS

Establishment of Routine Enzyme Assay Procedure

The conversion rate, which meant a percentage of each 5α-reduced steroid of the applied radioactivity, was 3–6% for 5α-androstane-3β,17β-diol, 5–10% for DHT and less than 2% for 5α-androstanedione when 50–100 μg of the sebaceous or sweat gland was used. As a result of recrystallization, the purity of the radioactivity tentatively identified as these metabolites was 75–95% (Table I). Besides, 0.5-2% of the applied radioactivity had the same mobility with androstenedione. Polar metabolites remaining at the origin accounted for about 3% of the recovered radioactivity. Thus, 80–95% of the radioactivity in the incubation mixture was recovered by extraction. In the blanks containing the incubation mixture only, the radioactivity corresponding to DHT acetate, 5α-androstane-3β,17β-diol diacetate or 5α-androstanedione was less than 0.5% of the recovered activity and further decreased to a negligible level after recrystallization.

The routine assay procedure was established from the results demonstrated in Fig 1 to 4. Sebaceous glands microdissected from a specimen of the nevus sebaceus of a 13-yr-old boy were used throughout these experiments. The pH optimum was about 7.5 (Fig 1). The time-course change in the appearance of 5α-reduced metabolites during the incubation is illustrated in Fig 2. The rate of 5α-reduction decreased to nearly one-half after 15 min and further to ¼ after 1 hr. Since incubation was usually carried out for 1 hr, the rate of 5α-reduction was underestimated about 35% compared with the rate during the first 15 min. The relation between the concentration of substrate testrosterone and the rate of formation of 5α-reduced steroids is given in Fig 3. The reaction reached a plateau when the concentration of testosterone was about 5×10^{-5} M. The apparent Michaelis constant was about 2.4×10^{-5} M as estimated from Lineweaver-Burk plots. Fig 4 shows the relationship between the tissue weight and the rate of formation of 5α-reduced metabolites. The rate was slightly reduced when tissues over 50 μg were used. Therefore, the routine assay was performed on 20–50 μg of microdissected tissues incubated with 2 μM ^{3}H-1,2-testosterone for 1 hr at 37°C.

Testosterone 5α-Reduced Activity of Various Skin Tissues

The rate of formation of 5α-reduced metabolites by various tissues microdissected from skin specimens in 12 individuals are summarized in Table II. The results are expressed on a dry weight basis. In addition, the content of DNA was measured in some of the skin specimens, since the enzyme is presumably localized in the cells. The DNA content of the epidermis and of its appendages did not differ significantly from each other, while that of the dermis was ½ to ¼ of them. Thus, the relative strength of 5α-reductase activity does not appear to differ significantly, whether it is compared on a dry weight or DNA basis, except in the dermis.

The 5α-reductase activity of the sebaceous gland ranged from 84.7 to 261 pmoles/mg/hr. The activity of the sebaceous gland from forehead or scalp skin collectively was 45% higher on an average than that from any other part than the head. Further studies would be needed to establish whether this difference is significant. The sweat glands of axillary skin, which are probably apocrine glands judging from yellowish color and large lumina, presented the highest activity in the skin. Eccrine sweat glands showed rather a high activity, but it may be too early to conclude that they possess a 5α-reductase activity comparable to that of sebaceous glands, since only 2 samples were included in the present study and, moreover, it is not always easy to differentiate eccrine from apocrine sweat glands. Hair follicles performed a low rate of 5α-reduction compared with those 2 kinds of glands described above. Whereas the dermis often actively converted testosterone to 5α-reduced metabolites, the epidermis failed to carry out an appreciable degree of conversion. The variation of the enzyme activity in the dermis as noticed by us may be ascribable to unavoidable contamination with different sorts of cellular components such as blood vessels, residual sweat glands and pilosebaceous units.

DISCUSSION

Our knowledge of the localization of 5α-reductase activity in the skin largely depends upon indirect evidence obtainable from a study with whole skin: the activity is high in genital [2] and axillary skin [4], and higher in the facial skin of acne-bearing subjects than in that of nonacne subjects [5]. Direct measurement of 5α-reductase activity was performed on plucked human hair follicles [14,15] and cultured skin fibroblasts [16]. However, in addition to complexity of the skin constituents, different experimental conditions used in these studies, which may possibly have affected the *in vitro* testosterone metabolism [17], does not permit us to draw a parallel between various components of the skin from different regions or those in a diseased state in terms of 5α-reductase activity. Recently Hay and Hodgins [10] described the distribution of 5α-reductase activity in human skin using tissues isolated from axillary and forehead skin by dissection with or without collagenase treatment.

Our routine assay procedure was established by the experiments with a tissue specimen of nevus sebaceus merely for the reason that it could provide an ample amount of dissected tissues. This time we had no other alternative but to use it, though what was found on this tissue might not necessarily be true of sebaceous glands at other sites or from other tissues.

Generally we confirmed the finding of Hay and Hodgins [10] that the enzyme activity is mainly localized on the sebaceous gland and sweat (probably apocrine) gland. Their study disclosed the localization of 5α-reductase activity but failed to provide an objective basis for comparison of the activity between the individual components, though this never impaired

TABLE I. *Recrystallization of ^{3}H-dihydrotestosterone acetate, ^{3}H-5α-androstane-3β,17β-diol diacetate and 5α-androstanedione to constant specific activity*

Steroid recovered	Tissue	Specific activity of metabolites (cpm/mg)			
		Original	1	2	3
5α-Dihydrotestosterone acetate	Sebaceous gland	425	417	401	416
5α-Dihydrotestosterone acetate	Sweat gland	2922	2591	2362	2386
5α-Androstane-3β,17β-diol diacetate	Sweat gland	2013	1827	1597	1553
5α-Androstanedione	Sebaceous gland	95.9	74.8	67.8	67.3

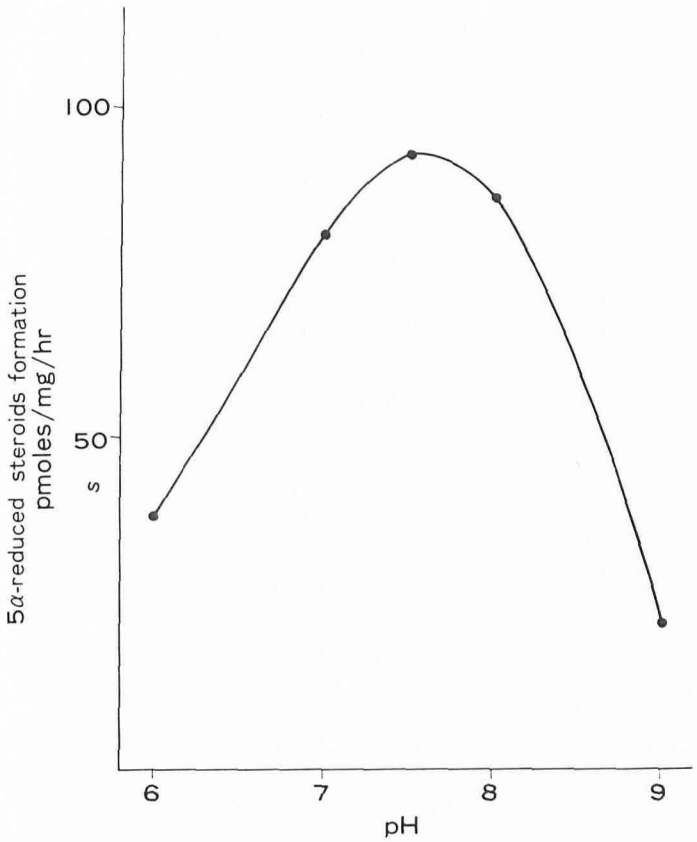

FIG 1. The effect of pH on the 5α-reduction of testosterone by human sebaceous glands. The experimental conditions are described in the text except for Tris buffer at pH 7–9 and HEPES buffer at pH 6. Sebaceous glands microdissected from a specimen of nevus sebaceus of a 13-yr-old boy were used as the enzyme source. This also applies to Fig 2–4. Each *point* is a mean of 3 determinations.

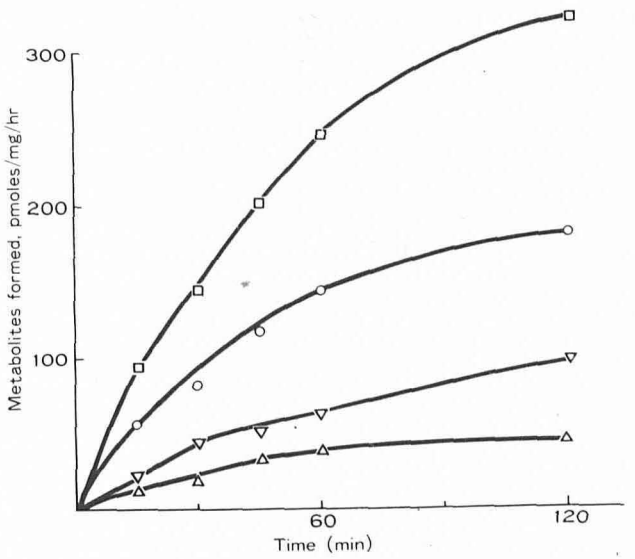

FIG 2. Time-course change in the formation of 5α-reduced metabolites from ^{3}H-testosterone by the sebaceous gland. Each *point* represents a mean of 3 determinations. ○——○ DHT; ▽——▽ 5α-androstane-3β, 17β-diol; △——△ 5α-androstanedione; □——□ total 5α-reduced metabolites (sum of the above 3 steroids).

the value of their work. They in fact expressed the conversion rate of testosterone without reference to tissue weight or DNA or protein content. In the present study, we quantitatively determined the enzyme activity in a given amount of tissues, thus making feasible a reasonable comparison for the first time. The high 5α-reductase activity in the sebaceous and the apocrine gland is in accord with the responsiveness of these glands to sex hormones and supports the view that in these glands

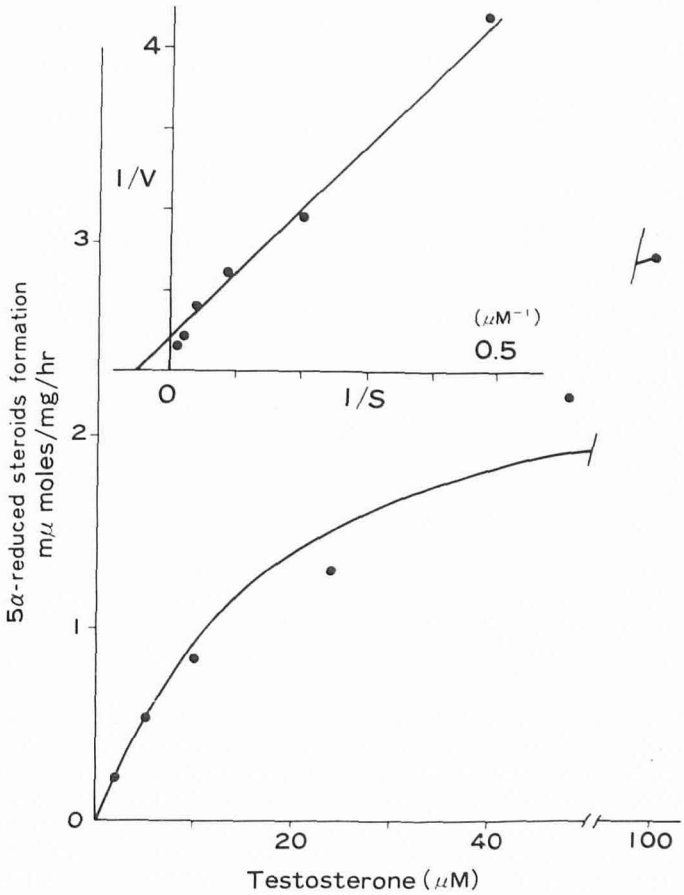

FIG 3. The effect of substrate concentration on the 5α-reduction of testosterone. Each *point* is a mean of 2 determinations.

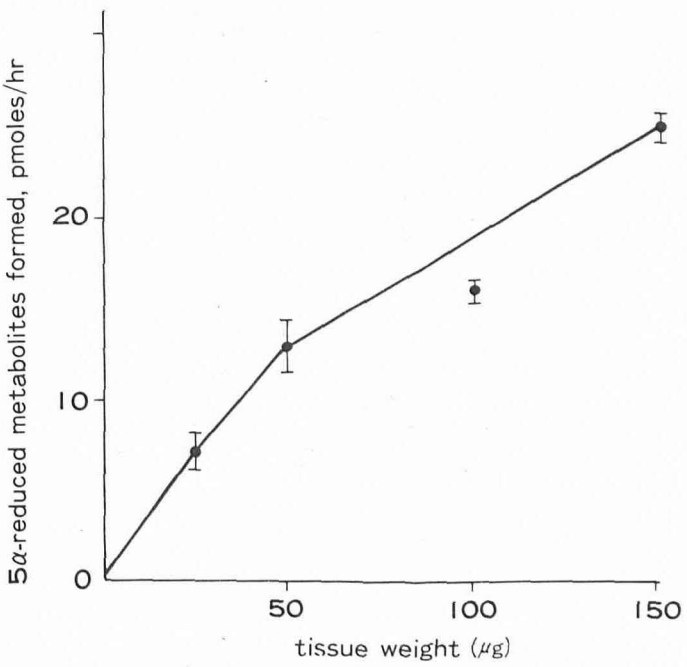

FIG 4. Testosterone metabolism as a function of tissue weight. Sebaceous glands were incubated with 2 μM of ^{3}H-testosterone for 1 hr. Each *point* indicates a mean and a standard error of 3 determinations.

TABLE II. *The rate of formation of 5α-reduced metabolites by various tissues microdissected from freeze-dried skin and their DNA content*

Tissue	Subject	Age	Sex	Region	5α-Reductase activity: pmoles/mg/hr	DNA content: ng/μg dry tissue
Sebaceous gland	A	17	M	Forehead	244 ± 7.84 (3)	—
Sebaceous gland	B	51	M	Forehead	123 ± 2.94 (3)	9.42
Sebaceous gland	C[a]	41	M	Forehead	180 ± 23.7 (3)	15.7
Sebaceous gland	D[b]	16	F	Forehead	161 ± 18.7 (4)	—
Sebaceous gland	E	26	F	Scalp	261 ± 54.2 (3)	—
Sebaceous gland	F[b]	13	M	Scalp	112 ± 10.5 (5)	15.7
Sebaceous gland	G	53	M	Back	84.7 ± 3.04 (3)	11.3
Sebaceous gland	H	24	F	Chest	104 (1)	—
Sebaceous gland	I	22	F	Axilla	141 (1)	—
Sebaceous gland	J	41	F	Axilla	126 (1)	—
Sebaceous gland	K	24	M	Prepuce	178 (1)	—
Sebaceous gland	L	48	M	Inguinal	112 (1)	—
Sweat gland	E	25	F	Scalp	221 (1)	14.5
Sweat gland	C[a]	41	M	Forehead	67.9 (1)	22.6
Sweat gland[c]	I	22	F	Axilla	399 ± 33.5 (4)	13.3
Sweat gland[c]	J	41	F	Axilla	377 ± 58.0 (4)	—
Hair follicle	I	22	F	Axilla	47.5 ± 5.9 (2)	—
Hair follicle	E	25	F	Scalp	33.4 ± 7.0 (2)	8.17
Epidermis	B	51	M	Forehead	negligible (3)	24.6
Epidermis	K	24	M	Prepuce	negligible (3)	—
Dermis	A	17	M	Forehead	23.6 ± 2.91 (3)	—
Dermis	B	51	M	Forehead	61.0 ± 18.1 (3)	7.23
Dermis	F[b]	13	M	Scalp	19.3 ± 5.3 (2)	—
Dermis	J	41	F	Axilla	29.8 ± 0.9 (2)	5.47
Dermis	G	53	M	Back	negligible (3)	—
Dermis	K	24	M	Prepuce	negligible (3)	—

Each value represents a mean and a standard error. The figure in parentheses shows the number of examined samples.
[a] Pachydermoperiostosis.
[b] Nevus sebaceus.
[c] Probably apocrine sweat gland.

androgens exert their effect by converting testosterone to DHT as in the prostate. The hair follicle gave a relatively low rate of 5α-reduction. Though assays were obtained from only 2 samples, it may be mentioned that the value was lower than that previously reported by us [14]. This difference may possibly be ascribable to the difference in the method of tissue removal and in substrate concentration.

The pH optimum in the isolated sebaceous gland was found to be about 7.5. It differs from the value of cultured fibroblasts from human genital skin, one of the typical androgen target tissues [16]. The latter produced a sharp peak of 5α-reductase activity at pH 5.5 and another subtle one near pH 9.0. The K_m of the enzyme in the sebaceous gland as determined in the present study is 10 times as great as that of cultured fibroblasts of genital skin, while being similar to the values reported of hamster sebaceous glands [18,19]. Whether such differences actually represent different enzyme characteristics in various target tissues remains to be elucidated, since the enzyme sources used in these experiments with both cultured fibroblasts and sebaceous glands were extremely crude.

The present finding of the formation of 5α-androstane-3β,17β-diol by isolated sebaceous glands is in agreement with the previous observation that this compound was one of the major metabolites of testosterone 5α-reduced *in vitro* by human scalp and back skin, both of which contain large sebaceous glands [20]. In addition, this finding upholds the previous suggestion based on the rat experiments in which this steroid was shown to stimulate sebum secretion [21,22].

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Announcement

The 22nd Annual Postgraduate Course in Dermal Pathology will be presented by the University of Texas Health Science Center at Houston, Division of Continuing Education and The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas, August 17–22, 1980.

The 22nd annual course in Dermal Pathology will be held August 17–22, 1980. During this 5½ day course, emphasis will be given to a review of the basic concepts of dermal pathology, including histology and anatomy, electron microscopy, histochemistry, inflammatory dermatoses, granulomatous dermatoses, reticuloendothelial and alternative dermatoses and nevi and neoplasms. The course is designed primarily for pathologists and dermatologists. The curriculum will be similar to that of previous courses which have been held in Houston, at the Skin and Cancer Hospital of Temple University in Philadelphia, and the University of California at Irvine. This will be the 7th time that the course will have been held in Houston. In addition to the didactic presentations emphasis will be given to microscopic slide study and self-evaluation examinations. This continuing medical education offering meets the criteria for 53 hr in Category 1 of the Physician's Recognition Award of the American Medical Association.

For further information please write to: The Office of the Director, The University of Texas Health Science Center at Houston, Division of Continuing Education, P. O. Box 20367, Houston, Texas 77025.