Table 1. Comparison of untreated and extracted albumin.

	Untreated albumin	Extracted albumin
Sedimentation constant, S_w20*	4.3	4.3
Electrophoretic mobility, descending boundary, $\mu \times 10^5$	6.50	6.52
Optical rotation, $[\alpha]_D^{25}$	−55.7	−53.7

* Not extrapolated to zero concentration; albumin concentration approximately 0.7 g/100 ml.

by the method of Gordon (*3*). Analysis revealed that it contained 1.3 moles of long-chain fatty acid per mole of albumin (*4*). The human and bovine mercaptalbumin preparations may, of course, differ significantly in this respect; unfortunately none of the afore-mentioned human mercaptalbumin preparation is available for analysis. When preliminary attempts, in this laboratory, to prepare human serum albumin free of long-chain fatty acids by using mixed-bed resins proved unsuccessful (*5*), the method described in this report was developed.

The human serum albumin used in this study was a sample of fraction V, produced by fractionation of pooled blood plasma by the method of Cohn *et al.* (*6*). Stabilizers (N-acetyl tryptophane or sodium caprylate) or heavy metals had never been added to this material, and it had received no heat treatment (*7*).

A water solution of serum albumin was lyophilized, and the dried albumin was covered with a mixture of 5-percent glacial acetic acid (by volume) in isooctane. The acetic acid-isooctane mixture had been pretreated with anhydrous Na_2SO_4 to remove traces of water. The extraction was carried out without agitation, at 0°C, for 6 hours or more. The extraction mixture was then decanted and discarded, the albumin was washed with an aliquot of isooctane, and the extraction was repeated in identical fashion. The albumin was then washed twice with aliquots of isooctane and was subjected to a vacuum for several hours in order to remove remaining isooctane and acetic acid.

When the resulting albumin preparation was dissolved in water, a turbid solution was obtained. The turbidity is caused, it is believed, by the presence of isooctane that has not been removed by the vacuum distillation. It could be made to disappear by any one of the following procedures: by dialysis against water; by allowing the solution to stand from 12 to 24 hours at room temperature or from 3 to 4 days at 1°C; or by application of a vacuum to the solution for a short period. The procedure selected was exhaustive dialysis of the albumin solution against distilled water. Acetic acid which remained was removed concomitantly during this dialysis; this was demonstrated by the disappearance of tracer amounts of C^{14}-labeled acetate that had been added prior to dialysis. The albumin preparation was then lyophilized and stored at −10°C.

Analysis of the serum albumin before extraction, by the method of Gordon, revealed that it contained 1.8 moles of long-chain fatty acid per mole of protein. After extraction, it contained only 0.02 mole of fatty acid per mole of albumin by the method of Gordon, and 0.10 mole of fatty acid per mole of albumin by the method of Dole (*8*).

The extracted, fatty acid-free albumin has been extensively compared with the untreated albumin to determine whether denaturation may have occurred during the extraction procedure. Some of the data obtained are presented in Table 1. Ultracentrifugal analysis was performed in a Spinco model E ultracentrifuge. Electrophoretic analysis was performed in barbital buffer, *p*H 8.6, ionic strength 0.1, in an Aminco model B electrophoresis apparatus. Optical rotation was measured in a Brinkmann polarimeter in which photocells from Rudolph and Sons were used. There was no detectable difference between the two albumin preparations by any of these analytic techniques.

The extracted albumin is also immunologically identical with the untreated albumin. This has been demonstrated by the agar diffusion method (*9*), with rabbit antiserum against recrystallized human serum mercaptalbumin. The "reaction of identity" was obtained at the junction of the precipitin lines for the two preparations. Finally, the two albumin preparations react identically with methyl orange anions. This has been demonstrated by a quantitative study of the binding of methyl orange anions to serum albumin by the method of equilibrium dialysis. Studies were performed with the untreated albumin, with the extracted albumin, and with a preparation of the extracted albumin to which 0.9 mole of oleic acid and 0.9 mole of palmitic acid had been added per mole of protein. The binding curves for all three preparations were identical, within the limits of error of the measurements. The accumulated evidence thus indicates that the albumin is not significantly altered during the extraction procedure.

In experiments designed to study the binding of small molecules or ions to serum albumin, an albumin preparation from which all tightly bound small molecules and ions have been removed should be used. The method described here was, in fact, developed in the course of quantitative studies of the interaction of human serum albumin with long-chain fatty acid anions. This method, which may be of general interest in investigations of the chemistry of albumin, may therefore prove to be of particular value in preparing albumin for use in a wide variety of binding studies.

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Sensitivity of the Skin to Changes in Rate of Intermittent Mechanical Stimuli

We have recently reported data on comparative speed of response of the eye and the ear, shown by measuring difference limens (DL) for intermittent stimuli (*1–3*). This paper (*4*) reports extension of such measurements to rate discrimination by the human skin and compares these with difference limens on flutter—that is, rate discrimination for intermittent white noise. The skin may be considered to be the phylogenetic antecedent of the ear, and v. Békésy's remarkable success in demonstrating the skin as a dimensional model of the cochlea points up the similarity of the two sensory processes (*5, 6*). It is shown in this report that data on rate discrimination suggest a further similarity between the skin and the ear.

The procedure was to measure differ-

ence limens for intermittent mechanical pulses, at pulse repetition rates of 1 to 320 cy/sec, by means of a phonograph cutting head in which the moving element was held between the thumb and one other finger. The moving element was fitted with a flat plate of 50 mm². Therefore, the total skin surface that was excited was 100 mm². Ten thresholds from each of five observers were obtained at each of ten frequencies between 1 and 320 cy/sec; electric pulse durations that were increased from 1.5 msec at 320 cy/sec to 7.5 msec at 1 cy/sec were used. The sensation level above the absolute threshold for feeling vibration was from 17 to 26 decibels.

Two electronic, rectangular pulse generators of variable frequency and pulse duration drove the mechanical stimulator. The observer felt the moving element of the vibrator on the skin of the finger tips of one hand and sampled the standard and comparison frequencies successively by a selector switch which he operated with the other hand. He also used his switch hand to control the frequency of the comparison stimulus by a linear multirevolution potentiometer. The experimenter set the comparison frequency away from the standard by a random amount, and the observer obtained equality judgments by the method of adjustment, alternately starting from frequencies above and below the standard. The discrepancy between the standard and the comparison frequencies was measured with an electronic counter, to an accuracy exceeding that of the generating equipment. The circuit that was required to produce the standard and comparison repetition rates and pulse durations is described elsewhere (*7*). The mechanical stimulator was mounted in a sound-absorbing box, and a masking noise was delivered to the observer through headphones.

Table 1. Difference limens for intermittent mechanical stimulation on the finger tips. Cutting head stimulator with 100 mm² contact area. Five observers.

Frequency (cy/sec)		*AD* (Δf) (cy/sec)	$\Delta f/f$ from *AD*	Cumulative DL's (*jnds*)
Standard	Mean obtained			
1	0.99	0.02	0.02	35.71
2.5	2.50	0.06	0.02	70.80
5	5.01	0.20	0.04	89.93
10	10.13	0.45	0.04	106.52
20	19.68	0.76	0.04	126.36
40	39.98	1.18	0.03	151.68
80	80.21	3.90	0.05	167.04
160	162.21	12.96	0.08	173.22
240	228.25	13.89	0.06	178.98
320	325.02	24.43	0.08	180.62

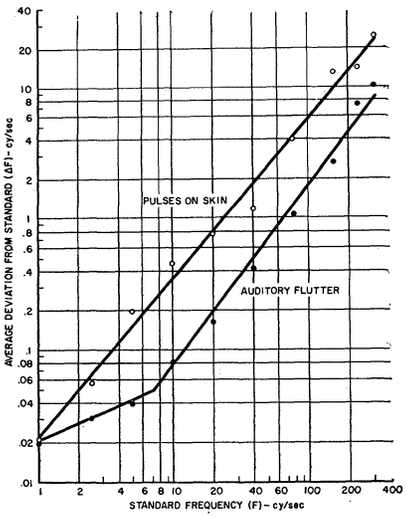

Fig. 1. The difference-limen function for discriminating rate. Upper curve: short pulses by the skin of the finger tip; log $\Delta f = (0.3410\text{-}2) + 1.2008 \log f$, for $f = 1$ to 320 cy/sec. Lower curve: intermittent white noise with a duty cycle of 0.5; visually fitted curve from 1 to 5 cy/sec; log $\Delta f = (0.5357\text{-}3) + 1.3479 \log f$, for $f = 5$ to 320 cy/sec.

The results for the rate discrimination of short pulses are shown in Table 1 and in the upper curve of Fig. 1. Column 2 of Table 1 shows the mean obtained frequency. By comparing these figures with those in column 1, it can be seen that the standard is matched, on the average, with an error of less than 12 cy/sec. Column 3 shows Δf as the average deviation (*AD*), computed with reference to the standard frequency (f). This rises monotonically with f. Column 4 gives the relative difference limens $\Delta f/f$, and column 5 shows the cumulative difference limens found by graphic integration of $1/\Delta f$, where Δf is the average deviation in column 3. The result is 180 just-noticeable differences (*jnd*) in the range of 1 to 320 cy/sec. The observed relationship between Δf and f is best expressed by the equation $\log \Delta f = (0.3410\text{-}2) + 1.2008 \log f$ and, therefore, may be approximated by a parabola of the form $y = ax^b$.

Two other types of tactile stimulators were used to obtain similar results. These were a modified loud-speaker motor and a precision miniature vibration generator (*8*). Data were also obtained, by the method described, with contactors that covered 1 mm² and 20 mm² of skin area on a single finger tip. There was no indication that the size of the area stimulated made any difference in the difference limens for rate discrimination.

It is clear, of course, that no mechanical transducer of the type used here will faithfully follow the pulse-wave form of the electric input. At higher frequencies, the stimulus applied to the skin approaches a sine wave. Périlhou (*9*), and others, have measured Δf for sine waves. He found that $\Delta f/f$ varies with intensity between 0.02 and 0.40. The determination of accurate values of Δf with sine waves offers special difficulties, because frequency and intensity tend to be confounded in the matches. This appears not to be the case with pulses at low frequencies. We have found, therefore, that the linear relationship in Fig. 1 begins to break down after about 320 cy/sec, when the transducer no longer follows the rectangular input.

The lower curve in Fig. 1 shows our difference limens for flutter (*2*). The two curves have nearly the same slopes between 10 and 320 cy/sec; this suggests that the mechanism for rate discrimination is the same for the skin and the ear. Our values for frequency discrimination and those obtained by v. Békésy (*5*) with his dimensional model of the cochlea, in which he substitutes the skin of the arm for the basilar membrane, are in reasonably good agreement (see his Fig. 20). However, the skin has by no means the temporal resolving power of the ear at moderate sensation levels. In the frequency range of 1 and 320 cy/sec, the skin achieves less than 200 just-noticeable differences, whereas the ear produces more than 500.

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1 April 1957

Cold-Resistant Oat Varieties also Resistant to Heat

Apparently Trabut (*1*) was first to indicate that red oats resist heat. His reference was to Algerian oats, which are similar morphologically to the Red Rustproof oat in this country. Several writers have agreed with early statements (*2*)