E. Roland Menzel,[1] *Ph.D.; Johannes Everse,*[2] *Ph.D.;*
Kathleen E. Everse;[3] *Timothy W. Sinor;*[4] *and Jim A. Burt*[4]

Room Light and Laser Development of Latent Fingerprints with Enzymes

REFERENCE: Menzel, E. R., Everse, J., Everse, K., Sinor, T. W., and Burt, J. A., **"Room Light and Laser Development of Latent Fingerprints with Enzymes,"** *Journal of Forensic Sciences*, JFSCA, Vol. 29, No. 1, Jan. 1984, pp. 99-109.

ABSTRACT: A very pronounced enhancement in detectability of reasonably fresh latent fingerprints is obtained when the ninhydrin method is combined with enzyme treatment. Specifically, trypsin and pronase were found to be effective, particularly the former. When ridge detail in room light remains inadequate even upon enzyme treatment, laser examination following zinc chloride treatment can produce strong additional enhancement to render such prints identifiable. Our results with older prints have been marginal to date. Reasons for this and directions for future study are discussed.

KEYWORDS: criminalistics, fingerprints, ninhydrin, enzymes, trypsin, pronase, zinc chloride, lasers

Since the late 1960s, ninhydrin, the universal reagent for aminoacids, has been the workhorse of chemical development of latent fingerprints on porous surfaces, such as paper. Although ninhydrin has several attractive features, namely low cost, simple application, and absence of requirement of sophisticated instrumentation, many individuals do not excrete sufficient aminoacids in their palmar perspiration to leave latent prints detectable with ninhydrin [*1*].

Several approaches have therefore been taken to enhance latent fingerprint detectability by modifications of the ninhydrin method. One such modification that has proven quite successful is the use of zinc chloride, following ninhydrin, together with laser examination [*2-5*]. Studies of chemical modifications of the ninhydrin molecule itself [*6*] show promise as well. Attempts, albeit with little success in the past, have also been made to enhance fingerprint development by solution application of ninhydrin and trypsin [*7*], contained together in solvents such as methanol, ether and so forth.[5] Indeed, our earlier cursory work in this area [*2*] was fruitless. Nonetheless, the underlying concept of using enzymes to hydrolyze proteins of the fingerprint residue in situ to free aminoacids that can then react with ninhydrin or its analogues remained attractive in principle.

In this article, we report on the application of two hydrolytic enzymes, trypsin and pro-

Received for publication 10 May 1983; accepted for publication 31 May 1983.
[1] Director, Center for Forensic Studies, Texas Tech University, Lubbock, TX.
[2] Professor, Biochemistry Department, Texas Tech University Health Sciences Center, Lubbock, TX.
[3] Research associate, Texas Tech University Health Sciences Center, Lubbock, TX.
[4] Laboratory assistants, Physics Department, Texas Tech University, Lubbock, TX.
[5] We are indebted to R. J. Hazen of the FBI Academy in Quantico, VA, for bringing these attempts to our attention.

nase,[6] to enhance latent fingerprint development. Earlier lack of success is readily understood if one considers the reaction of such enzymes with proteins, as schematically shown in Fig. 1. Proper enzyme activity is aided by elevated temperature, namely body temperature (approximately) and a humid environment. Also, enzymes tend to denature, that is, to loose their activity, in nonaqueous solvents. This was a major cause for earlier failures. Accordingly, we investigated enzyme application which conforms to proper reaction criteria.

Experimental Procedure: Fresh Prints

Trypsin

A number of fingerprints were deposited on paper, cut such that half of each latent print could be used as a control. The other half of each print was then dusted with trypsin powder, either by sprinkling liberal quantities of the powder over the paper or by application with a brush. Prints were fresh to two days old. The dusted print halves were then left to incubate, either in an oven at 37°C in a humid atmosphere or under ambient conditions (20 to 25°C and roughly 50% relative humidity). Incubation times were initially varied from a few minutes to 24 h. These print halves, together with their control counterparts, were then treated with ninhydrin and left to develop in ambient conditions for one day. The ninhydrin formulation was comprised of a saturated methanol solution which was then diluted with freon (1,1,2, trichloro-1,2,2 trifluoroethane) in the proportion of 20% methanol solution to 80% freon by volume. We had previously found that this formulation generally develops latent prints in ambient conditions in a few hours. Comparison of the trypsin-treated print halves with the control halves almost always showed a very dramatic increase, often by about an order of magnitude (determined by reflectance measurements), in development of the trypsin-treated halves. Figure 2 illustrates such an instance. Optimum trypsin incubation times varied from generally nearly instantaneous to occasionally on the order of 10 min, depending on the "strength" of the fingerprint deposit. Although incubation in ambient conditions was slower than incubation in the oven, comparable results were achieved with optimum incubation times ranging from generally nearly instantaneous to occasionally on the order of half an hour.

The above approach to enhancement of ninhydrin development by pre-dusting with trypsin had three objectionable features:

1. Overincubation with trypsin tended to smudge out fingerprint ridge detail.
2. Overincubation tended to produce substantial background coloration, since ninhydrin reacts to some degree with trypsin itself to produce the familiar purple color. This background was sometimes sufficiently strong to nearly obliterate latent prints. On the left half of Fig. 2, background coloration is visible, but not strong enough to be objectionable. We found that dusting of the paper produced stronger background coloration than sprinkling the powder onto the paper.

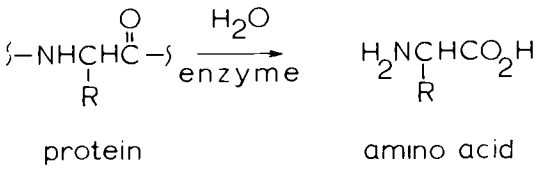

FIG. 1—*Reaction of enzyme with protein to form aminoacid.*

[6] Worthington, Biochemical Corp., Freehold, NJ and CalBiochem-Behring Corp., LaJolla, CA, respectively.

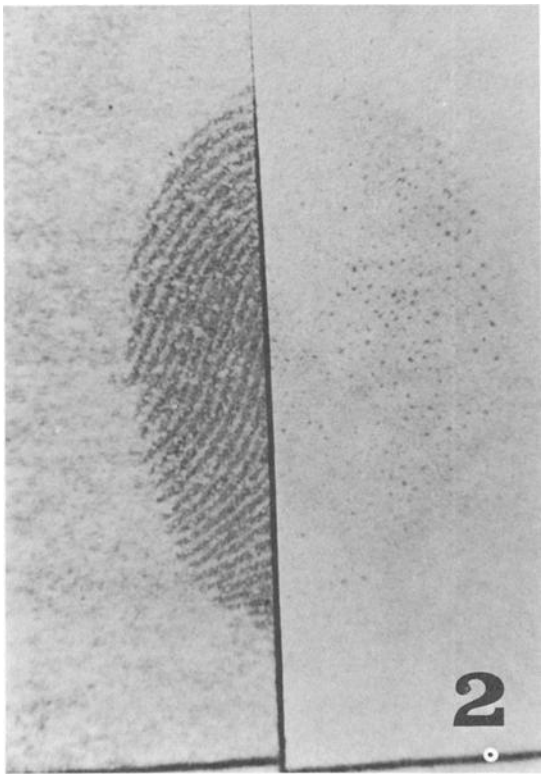

FIG. 2—*Room light photograph of latent print on paper treated with ninhydrin* (right half) *and trypsin/ninhydrin* (left half).

3. Optimum incubation times could not be determined beforehand for a given latent print.

We therefore reversed the order of fingerprint treatment. First, ninhydrin was applied to fingerprints on paper and the prints were left to develop for one day in ambient conditions. The prints were then cut in half and one half was dusted with trypsin as before, while the other half was retained as control for comparison purposes. Incubation times at ambient conditions of about one day were found to be needed to produce substantive enhancement of fingerprint development. Suitable incubation times in the oven were shorter, about 5 to 8 h.

Despite the increased incubation time requirement, the treatment with trypsin following ninhydrin was found to have a number of advantageous features:

1. If any ninhydrin development at all is discernible, one does not have to treat the whole article under scrutiny, but can, instead, concentrate on a specific area.

2. One can visually monitor the fingerprint development since the ninhydrin is already in place and reacts with aminoacids as they are formed by the action of the enzyme. This means that one can, at one's convenience, terminate the reaction by removing excess trypsin either by blowing the powder off or by spraying it off with a nonaqueous solvent that also denatures the enzyme.

3. Background coloration is very much less problematic with trypsin after ninhydrin than with trypsin before ninhydrin. The intensity of fingerprint development is lower as well with trypsin after ninhydrin, but still quite good.

4. Unlike in the case of trypsin before ninhydrin, fingerprint detail does not show as much tendency to smudge out.

Even though the combination of ninhydrin and trypsin generally produced very satisfying results in room light, we still encountered a great many instances in which developed fingerprint detail remained poor, as illustrated in Fig. 3*a*. Even though the left half of the print (treated with trypsin before ninhydrin) clearly shows increased development compared with the control right half (treated with ninhydrin alone), the detail in the left half is weak. We therefore followed with zinc chloride (using the same solvent system as for ninhydrin) and laser examination [*2*] to attempt further development enhancement. Figure 3*b* shows the print of Fig. 3*a* after this procedure, clearly showing the expected enhancement of the ninhydrin-treated right control half, and still stronger enhancement of the enzyme/ninhydrin treated left half. We found the kind of enhancement shown in Fig. 3*b* as compared to Fig. 3*a* to be a general feature in instances in which the room light development was poor, unless strong background coloration occurred. Because trypsin treatment after ninhydrin generally did not produce much background coloration, we performed laser examination after this treatment sequence. Figure 4 shows a typical result which compares the room light development (Fig. 4*a*) with the corresponding development under the laser after zinc chloride (Fig. 4*b*). The right print half is the control portion, the left print half the enzyme treated one.

For fingerprints that were well-developed in room light after ninhydrin/trypsin or trypsin/ninhydrin, *in which case the laser is not needed*, laser enhancement was weak or nonexistent. This has the following reason. Fingerprint fluorescence tends to be quenched by self-absorption or intermolecular energy transfer or both for highly developed prints whereas background fluorescense, resulting from ninhydrin trypsin and then zinc chloride reaction, is not quenched nearly as much (unless background coloration is severe). Thus, fluorescence contrast is far less than that of weakly developed prints (in absence of significant background coloration). In addition, zinc chloride treatment of highly developed prints needs to be very delicate to prevent smudging out (bleeding) of ridge detail. This is not a problem with weakly developed prints. In general, but particularly for latent prints to be examined by laser, the ninhydrin/trypsin treatment sequence seems preferrable. Fluorescence enhancements of about a factor of three (based on fluorescence intensity measurements) were fairly typical.

Pronase

The procedures used for application of pronase to latent fingerprints followed along the lines described above for trypsin. The results were similar to those obtained with trypsin, with the following features:

1. The pronase powder did not lend itself as well to dusting as the trypsin powder.

2. Incubation times for the pronase/ninhydrin sequence were generally quite short, as with trypsin. For the ninhydrin/pronase treatment sequence, they were generally roughly a factor of two longer than for the corresponding typsin procedure for incubation in the oven. Ambient incubation for the ninhydrin/pronase sequence was found to be extremely slow.

3. In room light, the pronase/ninhydrin sequence produced background coloration similar to that of the trypsin/ninhydrin procedure. Fingerprint detail smudging, though not as pronounced as with trypsin, occurred as well. Figure 5 shows a room light photograph of a pronase/ninhydrin treated print half (left) and the corresponding control half (right) treated with ninhydrin alone. Although background coloration is clearly strong, the fingerprint detail of the left print half is still good and strongly enhanced compared to the right half.

4. Fingerprint coloration was generally somewhat weaker in room light for pronase as compared to trypsin.

5. Quenching of fingerprint fluorescence, with treatment either by the pronase/ninhydrin or the ninhydrin/pronase sequence, was more problematic under the laser in presence of

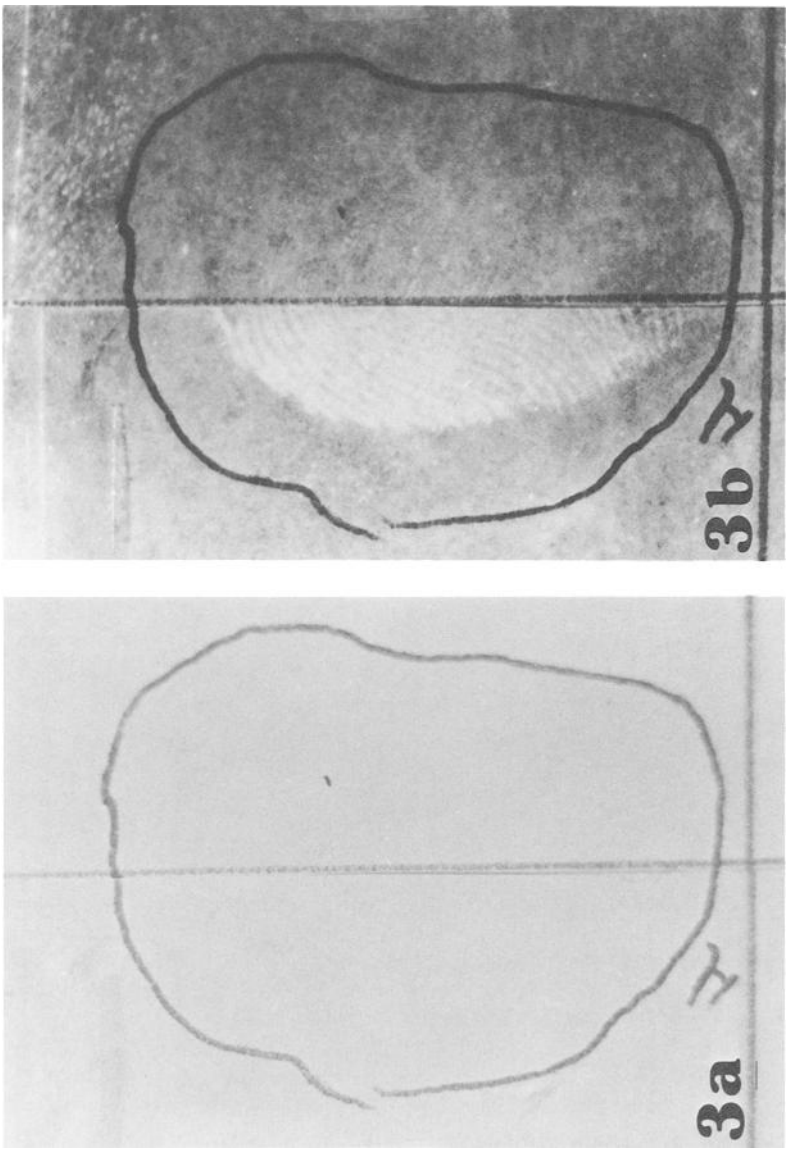

FIG. 3—*Comparison of room light photograph* (a) *of latent print on paper treated with the trypsin/ninhydrin sequence* (left half) *and ninhydrin* (right half) *with photograph of same under argon-ion laser illumination (blue-green) after zinc chloride treatment* (b).

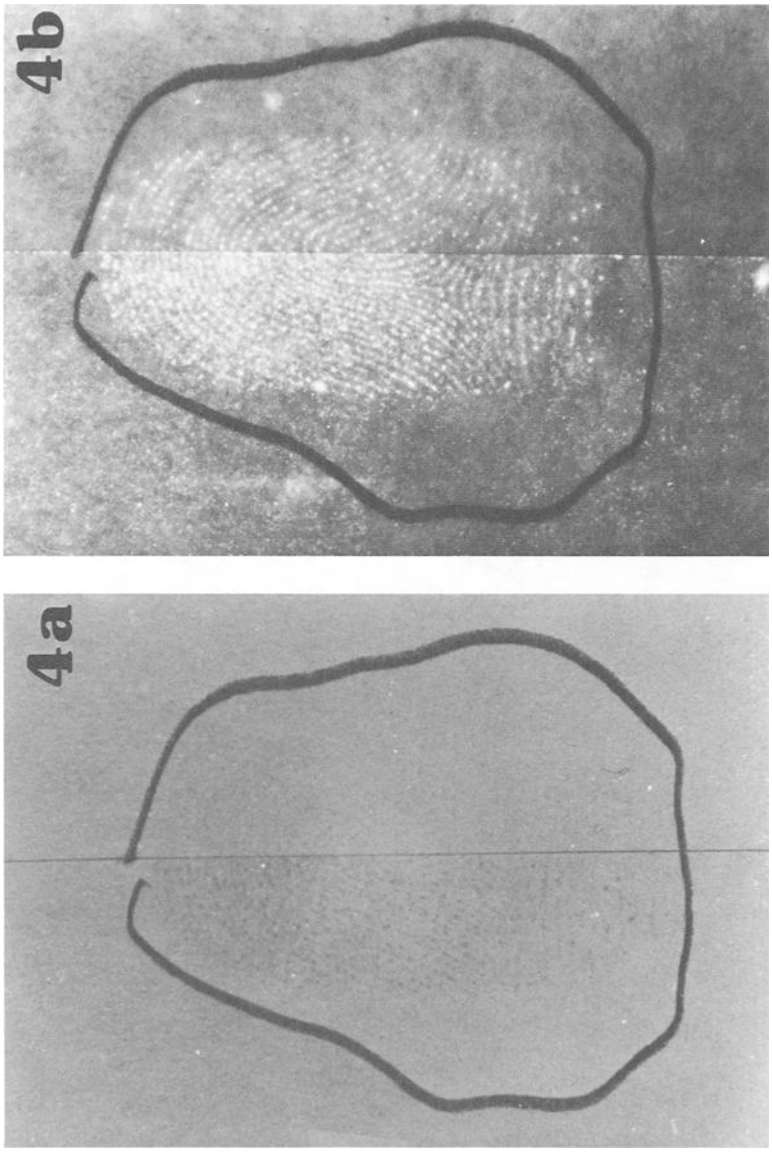

FIG. 4—*Comparison of room light photograph* (a) *of latent print on paper treated with the ninhydrin/trypsin sequence* (left half) *and ninhydrin* (right half) *with photograph of same under laser after zinc chloride* (b).

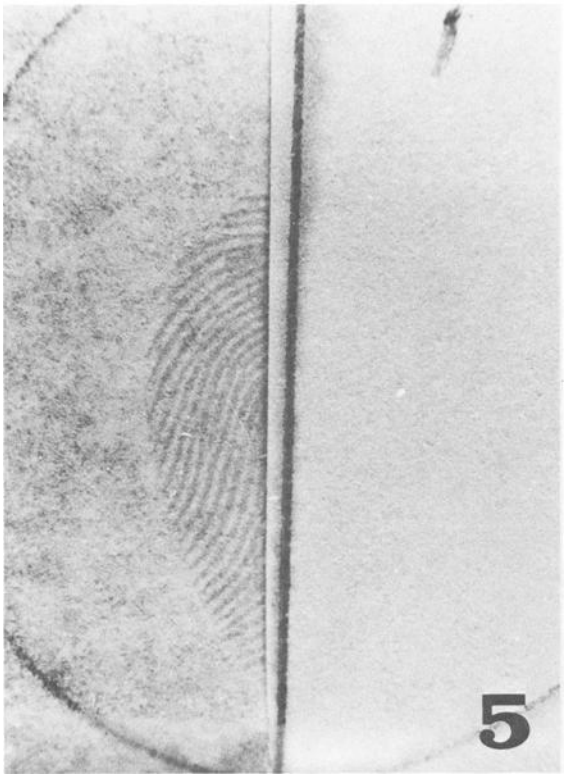

FIG. 5—*Room light photograph of latent print on paper treated with ninhydrin* (right half) *and pronase/ninhydrin* (left half).

background coloration than with the corresponding trypsin procedures. Thus, only very weakly developed prints in absence of substantial background coloration benefited from laser examination. Figure 6 shows such an instance. Figure 6*a* shows a ninhydrin/pronase-treated print (left half) and the control (right half) in room light, while Fig. 6*b* shows the same print on laser examination.

Experimental Procedure: Old Prints

General applicability of enzymes demands that they be able to enhance development of older latent prints, not just fresh ones. Accordingly, we applied the ninhydrin/enzyme and enzyme/ninhydrin sequences to latent prints about two weeks old in the manner described for fresh prints. The results had the following general features:

1. Incubation times for enhanced development in room light were longer than for fresh prints. Typically, one-day incubation in the oven was needed for the ninhydrin/trypsin sequence and on the order of one to several hours for the trypsin/ninhydrin sequence. Pronase yielded analogous results.

2. These incubation times yielded strong background fluorescence under the laser following zinc chloride treatment. With the ninhydrin/trypsin sequence, laser examination was generally useless. With the trypsin/ninhydrin sequence and incubation times on the order of half an hour, background fluorescence remained acceptably low and laser examination sometimes produced enhanced development. For incubation times of several hours, back-

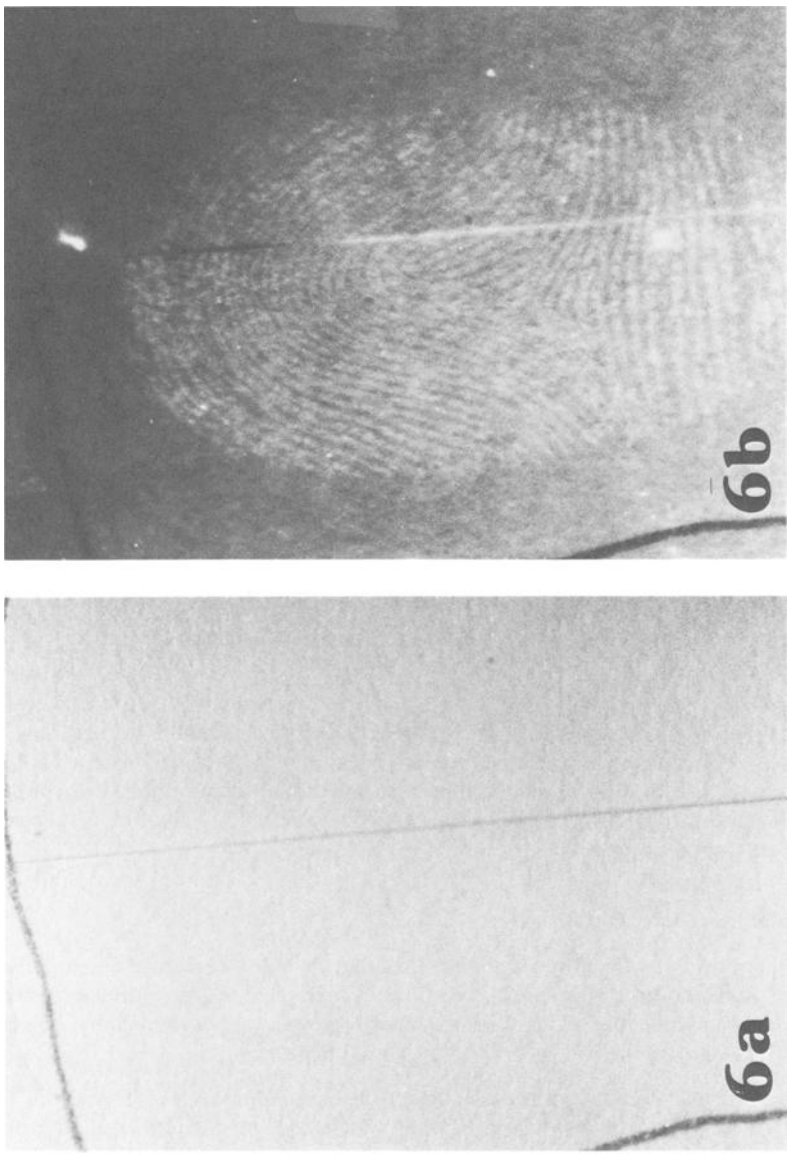

FIG. 6—*Comparison of room light photograph* (a) *of latent print treated with ninhydrin/pronase* (left half) *and ninhydrin* (right half) *with photograph of same under laser after zinc chloride* (b).

ground fluorescence became prohibitively strong. Pronase did not lend itself to laser examination.

3. Fingerprint development enhancement was often visible in room light, but not at all as pronounced as with fresh prints. Indeed, our results were generally marginal and critically dependent on incubation time, which could not be predicted beforehand.

Discussion

When trypsin (or pronase) is applied to fresh latent prints before ninhydrin, preferential adherence of the enzyme to the fingerprint residue is quite clearly visible. Indeed, trypsin constitutes a reasonably good dusting powder for fresh prints. The above described background coloration makes it clear that the enzyme, denatured once ninhydrin is applied, reacts with ninhydrin (in absence of protein hydrolysis) to form the familiar purple product. The background coloration obtained with the ninhydrin/enzyme sequence shows that the undenatured enzyme readily reacts with ninhydrin also. The possibility comes to mind that our results are not a result of protein hydrolysis, but simply of preferential enzyme adhesion to fingerprint residue and reaction with ninhydrin. One can easily explain our results on this basis for fresh prints with the enzyme/ninhydrin sequence. When the enzyme is applied after ninhydrin, sample inspection indicates that the enzyme no longer sticks preferentially to the fingerprint residue, or only slightly so. For older prints, where the fingerprint residue has lost water content and has penetrated into the paper, in comparison with fresh prints, no preferential adhesion at all of the enzyme to the fingerprint residue is apparent. Even for this situation, one might still interpret the results as follows based purely on enzyme/ninhydrin reaction. The ninhydrin at fingerprint ridge sites penetrates less deeply into the paper than between ridges and areas surrounding the print. Therefore, although the enzyme no longer adheres preferentially to the print, the enzyme/ninhydrin reaction remains preferential, if not as pronounced as with fresh prints.

To make a distinction between the above posed mechanism and the mechanism of protein hydrolysis by the enzyme, creating in situ aminoacids that react with ninhydrin we consider the following experimental observations. It has previously been found that latent print treatment by a combined solution of trypsin and ninhydrin in alcohol [*7*] or in methanol-freon mixture [*2*] produces no latent fingerprint development enhancement. This is easily understood in terms of protein hydrolysis because the enzyme is denatured by the alcohol. The absence of enhancement is rather more difficult to understand in terms of enzyme/ninhydrin reaction because the situation is essentially that of the ninhydrin/trypsin sequence discussed above, or, for that matter, the trypsin/ninhydrin sequence for older prints.

To obtain a conclusive determination, the following experiment was conducted. Fresh prints on paper were treated with the ninhydrin formulation cited earlier and left to develop for about 4 h in ambient conditions. Each print was then cut in half and one half was treated with trypsin as before while the other half was treated with denatured trypsin. The denatured trypsin was obtained by pouring trypsin powder into methanol and then letting the methanol evaporate. The trypsin-treated half prints were then incubated in the oven for 2 h. Good fingerprint development enhancement, with slight background coloration, was obtained with all samples. The denatured trypsin-treated half prints were incubated in the oven for 20 h to give these samples every chance to develop comparably to the trypsin-treated halves, particularly with respect to background coloration. We chose background coloration as a development criterion because the denatured trypsin formed a coarser powder than the undenatured trypsin, which would tend to reduce reaction rate. The background coloration (somewhat spotty at times) of the samples incubated for 20 h was comparable to or stronger than that of the samples incubated for 2 h, but in all cases the fingerprint development for the latter was stronger than for the former. Figure 7 shows an example. We consider this compelling evidence in favor of protein hydrolysis, particularly because of the following reason. It is known that the ninhydrin reaction with aminoacids is slow in ambient conditions.

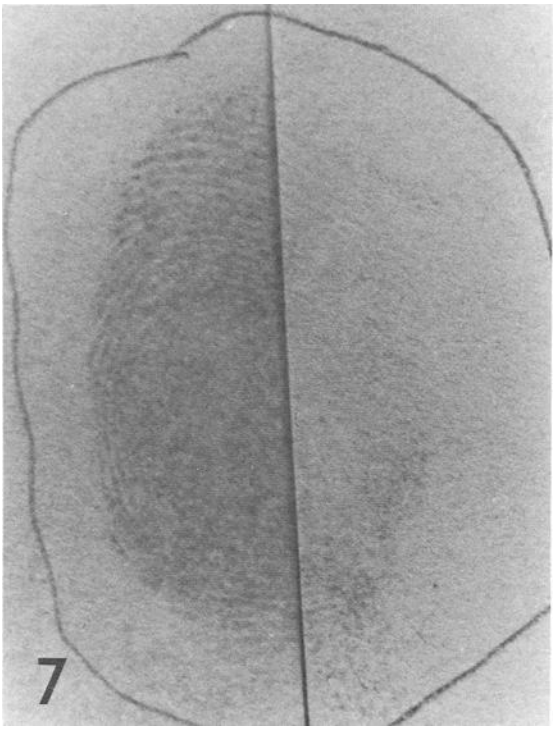

FIG. 7—*Room light photograph of latent print (on paper) treated with ninhydrin and then with trypsin* (left half) *and denatured trypsin* (right half). *See text for discussion.*

Therefore, heat together with moisture are often applied to expedite fingerprint development. One would thus expect that after 20 h versus 2 h of incubation, some fingerprint development improvement of the 20-h incubated samples might occur simply because of aminoacid/ninhydrin reaction in the customary fashion. In no case, however, did the 20-h incubated samples yield development competitive with the 2-h incubated samples.

For enzyme application to fresh prints, it is from a pragmatic standpoint not important whether the enzyme acts simply as a chemically activated dusting powder or whether protein hydrolysis takes place. Our results indicate that both mechanisms are operative for fresh prints in the enzyme/ninhydrin sequence, but we do not at present know to what relative extent. In the ninhydrin/enzyme sequence, our results indicate that the protein hydrolysis mechanism dominates. Our relatively marginal results with older prints are thus interpreted as a result of fingerprint residue penetration into the paper, with loss of water content, both of which cause enzyme activity inhibition. Since protein hydrolysis in fingerprint residue does take place, it should in principle be feasible to use enzymes for enhanced development of old prints, whereas without protein hydrolysis, enzyme application for this purpose would be useless.

We are now initiating investigation of a range of enzymes and enzyme application modes other than dusting to develop old latent prints, and we are beginning exploration of enzyme use for development of latent fingerprints on difficult surfaces, such as cloth and skin. Although much research remains to be done to bring enzyme use in fingerprint work to a mature state, our results to date imply much promise for this approach. If fresh prints are on hand, trypsin application should be valuable in case work even at the present stage.

Acknowledgment

This material is based upon work supported by the National Science Foundation under Grant CHE 8005515.

References

[1] Crown, D. A., "The Development of Latent Fingerprints with Ninhydrin," *Journal of Criminal Law, Criminology and Police Science*, Vol. 60, No. 2, June 1969, pp. 258-264.

[2] Herod, D. W. and Menzel, E. R., "Laser Detection of Latent Fingerprints: Ninhydrin Followed by Zinc Chloride," *Journal of Forensic Sciences*, Vol. 27, No. 3, July 1982, pp. 513-518.

[3] Menzel, E. R., "On the Development of Latent Fingerprints by Laser," *The Texas Lawman*, Vol. 50, No. 12, Dec. 1981, pp. 3-7.

[4] Menzel, E. R., "Laser Detection of Latent Prints: a Case Study," *Identification News*, Vol. XXXII, No. 2, Feb. 1982, pp. 3-6.

[5] Menzel, E. R., "Techniques for Latent Fingerprint Development: Lasers," *Campus Law Enforcement Journal*, Vol. 12, No. 2, March-April 1982, pp. 27-31.

[6] Almog, J., Hirshfeld, A., and Klug, J. T., "Reagents for the Chemical Development of Latent Fingerprints: Synthesis and Properties of Some Ninhydrin Analogues," *Journal of Forensic Sciences*, Vol. 27, No. 4, Oct. 1982, pp. 912-917.

[7] McLaughlin, A. R., "Developing Latent Prints on Absorbent Surfaces," *Fingerprint and Identification Magazine*, Vol. 42, No. 8, Feb. 1961, pp. 3-16.

Address requests for reprints or additional information to
E. R. Menzel
Center for Forensic Studies
Texas Tech University
Box 4180
Lubbock, TX 79409